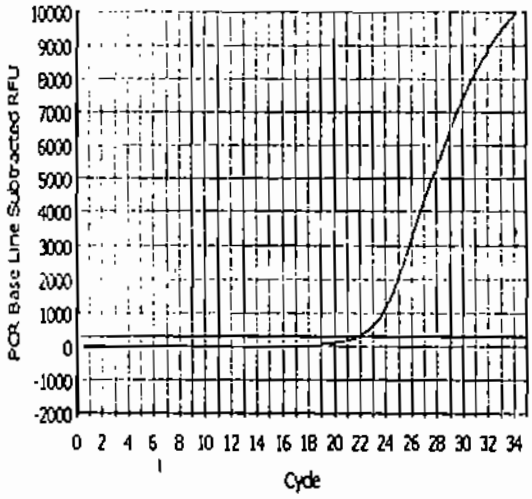
BEL 711 Recombinant DNA Technology
Major Exam IInd semester 2007-08

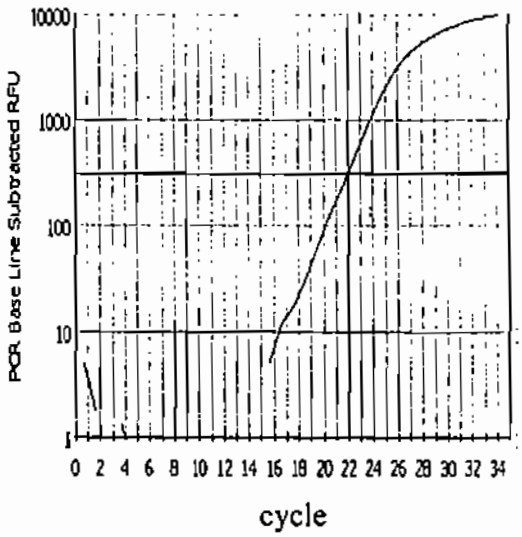
Time:2 hours Max Marks: 40

1. a) What is the problem with Taq DNA polymerase used for PCR? How can it be solved? 1 +1
b) What is Hot Start PCR? What advantage(s) can be derived from this strategy? 1.5 + 1.5
c) What is the minimum estimate of the length in base pair of a random primer for it to occur not more than once in human genome ($3.3X10^9$bp)? How do you derive it? 1.5+ 1.5

2. a)How sequencing by 454FLX system is a deviation from traditional principle of DNA sequencing? Explain its basic principles and operation **briefly but clearly**, with the help of a figure. Will the use of dideoxynucleotide analogues be practicable for 454 sequencing? Explain with convincing argument. What is the trade off in 454 sequencing? 2 + 2 + 1 + 1
b) 'Unlike 454FLX system, parallel sequencing by automated DNA sequencing of ABI PRISM system is not possible'. Justify in brief with crisp arguments either for or against it. 2

3. a) What are the traditional methods of quantitation of RNA? Why PCR is considered better than these methods? Why RT-PCR is better than PCR in this respect? 3
b) Why standards are necessary in RT-PCR? Give some examples of standards? Are standards necessary in the case of Relative RT-PCR? Explain briefly. 3
c) In the figures below results of same experiments are plotted. Why do they look different? What the thick horizontal line all way across represent? Between which cycles the quantitation should be restricted? 2





4. a) Given the role of specific hydrogen bond formation between restriction enzymes and their cognate recognition sequences, comment on how these interactions might be affected by pH, temperature and presence of salts. 2

b) Describe briefly the difference between specific and non-specific binding of restriction enzymes on the DNA. 2

c) Give two uses for DNA methylases with suitable examples. 2

5. Describe schematically the cloning strategy in cosmid vectors. Why is it necessary to do *in vitro* assembly of viruses during this proeedure? 3

b) The rarest mRNA in a cell of a particular tissue type has a concentration of five molecules per cell. Each cell contains 4,50,000 mRNA molecules. A cDNA library is made from mRNA isolated from this tissue. How many clones need to be screened to have a 99% probability of finding at least one recombinant containing a cDNA copy of the rarest mRNA? 3

6. Describe the structural features of a yeast integrative vector. Show schematically the events occurring at chromosomal level during its integration. How will you detect the presence of the specific gene (that has been cloned using an integrative plasmid) in the transformed yeast strain. 4