BITS, PILANI – DUBAI
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2009 – 2010
BIOT C418 GENETIC ENGINEERING TECHNIQUES
COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 2 hours **Date: 28.12.2009** **Max. Marks: 20**

Note:

a. Answer all the questions in the order

b. Draw schematic diagrams if required and answer to the point

1. What is the principle involved in the DEAE cellulose and Silica matrices used for the isolation of plasmid DNA. Explain the methods with suitable diagram. [1.0+1.0=2.0]
2. How the Glutathione-S-transferase fusion is used in the protein purification applications. Explain in detail with suitable diagram. [1.0]
3. What are the requirements for protein expression in *E. coli* host? [1.0]
4. How the T3 and T7 bacteriophage promoters are designed and utilized in the protein expression hosts of *E. coli*. Draw the mechanism with a suitable diagram. [1.0]
5. Explain the following with suitable diagram in detail. [1.0+1.0=2.0]
   (a) Genome mapping with restriction enzymes and
   (b) Genome mapping applications.
6. How polymerases are used to introduce mutations in the amplified gene product? Explain the following. [1.0+2.0=3.0]
   (a) Primer match
   (b) Addition of restriction enzyme sites
   (c) Point mutations by Oligonucleotide design and Altered Sites based mutagenesis
   (d) Methylated and uridylate-containing templates.
7. What are degenerate PCR primers? Explain any two applications of the degenerate PCR primers in Genetic Engineering Techniques. [2.0]
8. What are reverse transcriptases? [1.0]
9. Explain the following with suitable diagram. [2.0]
   (a) cDNA synthesis
   (b) RT-PCR
   (c) Primer extension
   (d) Mapping the 3' end of an RNA transcript.
10. Briefly explain the PCR cycle efficiency with respect to [3.0]
    (a) Perfect cycle (100% efficiency for PCR cycles) and
    (b) Simple predictive calculation.
11. How the genetic engineering techniques is used to generate [2.0]
    (a) Genetically modified crops and its applications
    (b) Genetically modified animal and its applications.

BITS, PILANI – DUBAI
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2009 – 2010
COURSE No: BIOT C418
GENETIC ENGINEERING TECHNIQUES TEST-II (OPEN BOOK)

Duration: 50 min. **Date: 13.10.2009** **Max. Marks: 15**

Note:
a. Answer all the questions
b. Answer to the point

1. The bacteriophage based vector (cosmid) was used to clone a 30kb DNA fragment and after ligation the cloned DNA was packaged into the bacteriophage lambda capsid.
   a. How will you increase the number of successful entry of the bacteiophage lambda containing the foreign DNA into *E. coli.*
   b. Explain with a suitable diagram with your reason. [2+2=4.0]

2. A thermophilic bacteria was isolated from the high temperature natural habitat (hot spring) in order to search for the high efficiency thermostable DNA Polymerases. The bacterium which was identified as the *Thermus aquaticus* sp., was tested for the plasmid profile which showed three bands on agarose gel electrophoresis. The same DNA was tested for the restriction enzyme digestion with *Sma*I which gave a smear (a series of multiple bands). Upon transformation of the plasmid DNA isolated from the *Thermus aquaticus* sp., was used to transform an aliquot of competent cells of *E. coli* and the plasmid was isolated from the few successful transformants of *E. coli* (unlike any other plasmid vectors which used to give a lawn of transformants) which upon restriction digestion with *Sma*I generated only one distinct band on the agarose gel which is a clear indication of the presence of only one plasmid instead of three different plasmids in the isolate *Thermus aquaticus* sp. The result also demonstrated that the *Ori* of the plasmid was recognized by the *E. coli*. The plasmid DNA from clone (the new plasmid introduced into *E. coli*) was isolated again and used for transforming the another sample of the same set of competent cells used for the first step (transformation of the plasmid isolated from the *Thermus aquaticus* sp., to *E. coli*) which showed high efficiency resulting a lawn of transformants in the LB agar plates.
   a. Reason why the isolated plasmid DNA of *Thermus aquaticus* sp., have shown a smear in the agarose gel [1.0]
   b. Why the plasmid DNA isolated from the *Thermus aquaticus* sp., was not efficiently taken up by the competent cells of *E. coli.* [1.0]
   c. Reason out why the new plasmid which was isolated from the *E. coli* resulted in a single band instead of three or more multiple bands in the agarose gel. [1.0]
   d. Why the same plasmid DNA transformed into *E. coli* and isolated from the same *E. coli* have shown high efficiency transformation. [1.0]
   e. Will you expect the expression of the protein, the thermostable DNA polymerase in the new host, the *E. coli* from the plasmid although it is recognized by the foreign host? If not how will you make the protein, the DNA polymerase of your new isolate in a foreign host *E. coli* for commercialization. [2.0]

3. Why the restriction digestion of the genomic DNA isolated from the *E. coli* with *Eco*RI show a smear on the agarose gel?
    a. Reason with the DNA fragments generated.
    b. What are the uses of such small fragments of DNA? Briefly explain. [1.0]

4. What are the different parameters which will be taken into account in choosing a polymerase for PCR? Briefly explain. [1.0]

5. What are the different parameters will you take into account while designing & handling forward and reverse primers in the laboratory for amplifying gene using PCR? Briefly explain. [1+1=2.0]

6. Reason out why do we need to add more restriction enzymes during setting up of DNA digestion reaction than the normally required quantity? [1.0]

BITS, PILANI – DUBAI
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2009 – 2010
COURSE No: BIOT C418
GENETIC ENGINEERING TECHNIQUES TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 25.10.2009** **Max. Marks: 15**

Note:
a. Answer all the questions
b. Answer to the point

1. Explain with a diagram for the importance of restriction enzyme selection in DNA cloning with respect to *Bam*HI and *Bgl*II. [2.0]

2. How the high salt concentrations affect the electrophoretic pattern of DNA on agarose gel? [1.0]

3. What are "high melting point" and "low melting point" agarose? Mention its applications. [1.0]

4. How the percentage agarose gel affect DNA electrophoresis? Explain. [1.0]

5. Why the horizontal agarose gel electrophoresis is often called "submarine gel"? What are the advantages of the submarine gels? [1.0]

6. How the PAGE (Polyacrylamide gel electrophoresis) is useful in DNA analysis? Compare with agarose gel electrophoresis. [2.0]

7. Compare TAE (Tris-Acetate-EDTA) and TBE (Trise-Borate-EDTA) buffers for DNA electrophoresis. [2.0]

8. Why salts are frequently used during DNA precipitation? Name any two salts with concentration used. [1.0]

9. Compare the plasmid DNA isolation by [2.0]
   a. Alkaline lysis
   b. Silica column
   c. Ion exchange and
   d. Cesium Chloride density gradient centrifugation

10. What are fusion proteins? Give at least 4 examples. [2.0]

Solution

BITS, PILANI – DUBAI
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2009 – 2010
COURSE No: BIOT C418
GENETIC ENGINEERING TECHNIQUES QUIZ-II (CLOSED BOOK)

Duration: 20 min. Date: 25.11.2009 Max. Marks: 5

Name: ID No:

1. Why the restriction enzyme digested vector (linearized) used for cloning experiment is dephosphorylated? Name the enzyme used for dephosphorylation. [1.0]

✓To prevent self ligation; phosphatase

2. What is TOPO technology? [1.0]

✓DNA ligation using topoisomease of vaccinia viru for cloning PCR products; the enzyme catalyze the reaction through DNA 3'-phosphotyrosyl enzyme intermediate; 3' phosphate to 5'-hydroxyl group.

3. What are shuttle vectors? Mention its applications. [1.0]

✓Have multiple origin of replication; to transfer from one host to another

4. Briefly discuss the different factors which influence the Restriction enzymes. Define enzyme unit. [1.0]

✓Temperature; pH; ionic strength; buffer concentration; enzyme unit: the amount of enzyme required to digest 1ug of standard DNA completely at 37°C in a 50uL volume.

5. Mention at least four methods by which the restriction enzymes are inactivated? Reason why inactivation is important in DNA cloning experiments. [1.0]

✓Heat inactivation; EDTA; phenol/chloroform extraction; gel elution by ion exchange; to minimize the interference of the protein with other procedures including ligation.

BITS, PILANI – DUBAI
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2009 – 2010
COURSE No: BIOT C418
GENETIC ENGINEERING TECHNIQUES QUIZ-I (CLOSED BOOK)

Duration: 20 min. Date: 7.10.2009 Max. Marks: 5

Name: ID No:

1. What are the general features of a cloning vector? [1.0]

2. How will you recognize a true clone (recombinant DNA) from the plasmid backbone? Mention at least 4 methods. [1.0]

3. How the modifications of a plasmid by the host cell affect recombinant plasmid? [1.0]

4. How a clone may get contaminated? Mention at least 4 sources of contamination? [1.0]

5. How the DNA is resolved and what are the parameters that determine the separation of DNA by using electrophoresis? [1.0]