

**BITS PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**COMPREHENSIVE EXAMINATION (CLOSED BOOK)**

**Duration: 3Hrs.**

**Date: 5.6.2013**

**Max. Marks: 40**

**Note:** *Answer all the questions*

*Answer to the point and draw suitable diagrams if required*

**PART-A**

1. a). How the restriction length polymorphisms are studied using DNA samples? Explain with suitable diagram on the principles and mention the applications of RFLP. [2.0]  
  
b) How RFLP analysis is different from microsatellite based DNA analysis? Briefly explain. [2.0]
2. What are the limitations of agarose gel electrophoresis? Explain with advances in the area of contour-clamped homogeneous electrical field (pulsed field gel electrophoresis). [1.5]
3. What are stringent washing in hybridization technique? Explain the principle with a suitable diagram. [1.5]
4. a). Why genomic DNA libraries are required and explain with respect to cDNA library? [1.5]  
  
b). Explain the Maniatis strategy for making gene library and any one method for cDNA library. [2.0]
5. Explain the dideoxy nucleotide based DNA sequencing method with principle, methods, detection systems and advances in automated DNA sequencing. [3.0]
6. Why gram positive cloning and expression systems are required? Explain any one gram positive system and the prerequisites for using such systems in recombinant DNA technology. [2.0]
7. How the *lac* and *tet* repressor based expression are used in eukaryotic systems? Explain with the principle involved, methods and application with schematic diagrams. [3.0]
8. Why the monoclonal antibodies are preferred over polyclonal and give any two examples each for transgenic animal and transgenic plants derived therapeutic proteins. [1.5]

## **PART-B**

Q9.(i) Most of the cloning strategies do not work for PCR amplified DNA, Justify. Briefly explain all the possible strategies that can help in cloning such DNA. (2)

(ii) Why do you need to ensure the Quality Control of RE used in cloning. Briefly discuss the procedures used in doing so? (2)

Q10.(i) What are important features of a modern plasmids .List out the major limitations of using Plasmid vectors . (2)

(ii) What is a litmus Vector? What are its properties? Explain. (2)

(iii) Briefly comment on the diagram given below and the concept involved. (2)

Q11. (i) Briefly describe structure and organization of the Ti plasmid and mechanism of gene transfer to plants . (2)

(ii) Can Agrobacterium mediated gene transfer be used to transfer genes in all types of plants, if yes, Why and if no, give the reasons. How can you use the system to transfer genes in such crops? (1)

Q12.(i) Write a Brief note on Map of Lambda chromosome . Wild type phage lambda contains several sites for Restriction endonuclease and therefore not suitable as vectors, Limiting the choice of sites for insertion of DNA. How the lambda phage is used as a vector in that case? Explain with an example. (2)

(ii) How is the copy number of the plasmids controlled/regulated? Explain (2)

Q13. a. What is the difference between transfection and transduction? (1)

b. What are early and late function genes? Which of these is the large T antigen? (1)

c. What are the characteristic advantages of using Adeno-associated Viruses as vectors? (1)

Ans key.

**BITS PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**COMPREHENSIVE EXAMINATION (CLOSED BOOK)**

**Duration: 3Hrs.**

**Date: 5.6.2013**

**Max. Marks: 40**

**Note:** *Answer all the questions*

*Answer to the point and draw suitable diagrams if required*

**PART-A**

1. a). How the restriction length polymorphisms are studied using DNA samples? Explain with suitable diagram on the principles and mention the applications of RFLP. [2.0]  
dna restriction fragments, difference in pattern of migration, species identification, mutations, polymorphism.  
b) How RFLP analysis is different from microsatellite based DNA analysis? Briefly explain. [2.0]  
differ in length due to number of copies, 1-12 nt in length, alleles, PCR products differ in size, restriction fragments.
2. What are the limitations of agarose gel electrophoresis? Explain with advances in the area of contour-clamped homogeneous electrical field (pulsed field gel electrophoresis). [1.5]  
Size, separation, resolution, large dna fragments, migration, pulsed field gel electrophoresis, greater than 200kb dna, orientation, sybr safe dna stains over ethidium bromide.
3. What are stringent washing in hybridization technique? Explain the principle with a suitable diagram. [1.5]  
Low stringency- allow partially complementary sequences for hybridization  
High stringency- only completely complementary sequences can bind to target  
Normally stringency washes are performed under successively more stringent conditions lower salt or higher temperature.
4. a). Why genomic DNA libraries are required and explain with respect to cDNA library? [1.5]  
cDNA are cell and tissue specific expressed genes whereas the genomic dna represent entire genome.  
b). Explain the Maniatis strategy for making gene library and any one method for cDNA library. [2.0]  
dna fragment, vector, lambda charan, methylase block RE sites, packaging invitro, and phage carries the dna fragments.
5. Explain the dideoxy nucleotide based DNA sequencing method with principle, methods, detection systems and advances in automated DNA sequencing. [3.0]  
ddNTP termination, pcr products, varying size, gel separation, Urea, high temperature, densitometer, fluorescent dyes, capillary gel electrophoresis, PHRED algorithms for profiling.
6. Why gram positive cloning and expression systems are required? Explain any one gram positive system and the prerequisites for using such systems in recombinant DNA technology. [2.0]  
B.cereus, protein solubility, shuttle vectors, e.coli.

7. How the *lac* and *tet* repressor based expression are used in eukaryotic systems? Explain with the principle involved, methods and application with schematic diagrams. [3.0]

Lac/tet repressor proteins, on/off system, cell lines, antibiotic selection, advantages of using phage promoters for constitutive expression of repressor, toxicity, controlled expression.

8. Why the monoclonal antibodies are preferred over polyclonal and give any two examples each for transgenic animal and transgenic plants derived therapeutic proteins. [1.5]

Specificity, antitrypsin, adenosine deaminase.

## PART-B

Q9.(i) Most of the cloning strategies do not work for PCR amplified DNA, Justify. Briefly explain all the possible strategies that can help in cloning such DNA. (2)

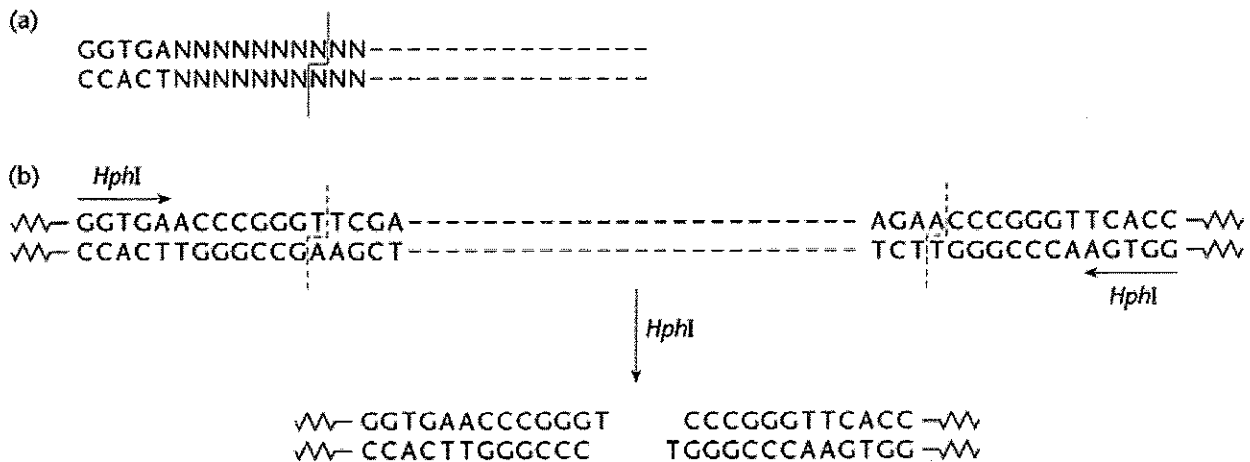
**Ans.** Most of the cloning strategies do not work for PCR products, since the polymerase used in the PCR have a terminal transferase activity.

Eg: the *Taq* polymerase adds a single 3' A overhang to each end of the PCR product. The PCR products cannot be blunt end ligated unless the ends are first *polished* (blunted).

A DNA polymerase like Klenow can be used to fill in the ends.

Alternatively, *Pfu* DNA polymerase can be used to remove extended bases with its 3' to 5' exonuclease activity.

One solution to the problem is to use T/A cloning. In this method, the PCR fragment is ligated to a vector DNA molecule with a single 3' deoxythymidylate extension.



Cleavage of a vector DNA molecule to generate single thymidylate overhangs.

The recognition sequence and cleavage points for the restriction endonuclease *HphI*.

Sequences in the vector DNA which result in desired overhangs after cleavage with *HphI*.

- (ii) Why do you need to ensure the Quality Control of RE used in cloning. Briefly discuss the procedures used in doing so? (2)

**Ans.** Possible contaminants:

Endo and Exonucleases- Exonucleases can cleave away overhangs and effect recombination.

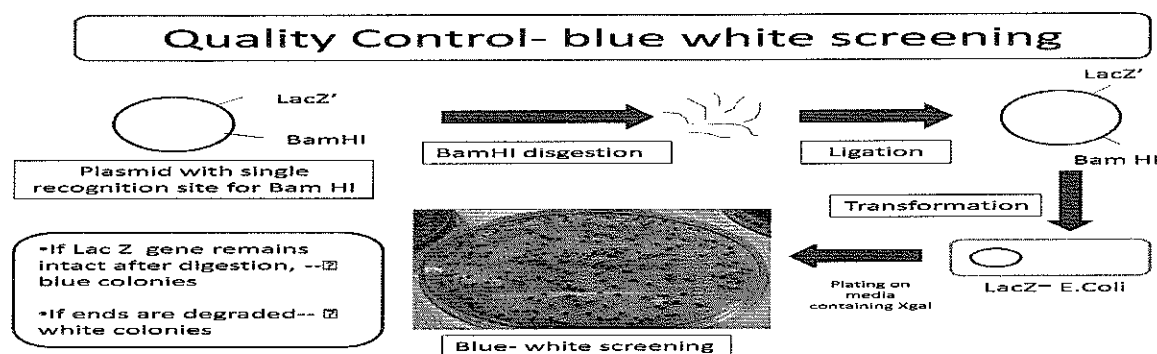
Phosphatases- May remove terminal phosphates and prevent ligation

QC Procedures:

DNA fragments are produced by excessive overdigestion of substrate DNA with RE .

Fragments are relegated Only occurs if 3' and 5' ends are intact and

Redigestion with same RE: Perfectly restored recognition sites will be re-cleaved to give the same banding pattern, indicating purity of enzyme



Q10.(i) What are important features of a modern plasmids .List out the major limitations of using Plasmid vectors . (2)

### Features: Small size

Origin of replication ,Multiple cloning site (MCS) ,Selectable marker genes

Some are expression vectors and have sequences that allow RNA polymerase to transcribe genes , DNA sequencing primers

Major Limitations “:Upper limit for clone DNA size is 12 kb

Requires the preparation of “competent” host cells

Inefficient for generating genomic libraries as overlapping regions needed to place in proper sequence

Preference for smaller clones to be transformed

If it is an expression vector there are often limitations regarding eukaryotic protein expression

(ii) What is a litmus Vector? What are its properties? Explain. (2)

**Ans. Litmus Vector :** A vector in which Polylinker regions flanked by two modified T7 RNA polymerase promoters

Each contains a unique restriction site (*SpeI* or *AflII*) that has been engineered into the T7 promoter consensus sequence so cleavage with the corresponding RE inactivates that promoter.

Both promoters are active despite engineered sites.

### Properties:

Blue/white screening can be used to distinguish clones with inserts from those containing vector only.

The LITMUS polylinkers contain 32 unique restriction sites. 29 leave 4-base overhangs and 3 leave blunt ends. The 3 blunt cutting enzymes have been placed at either end of the polylinker and in the middle of it.

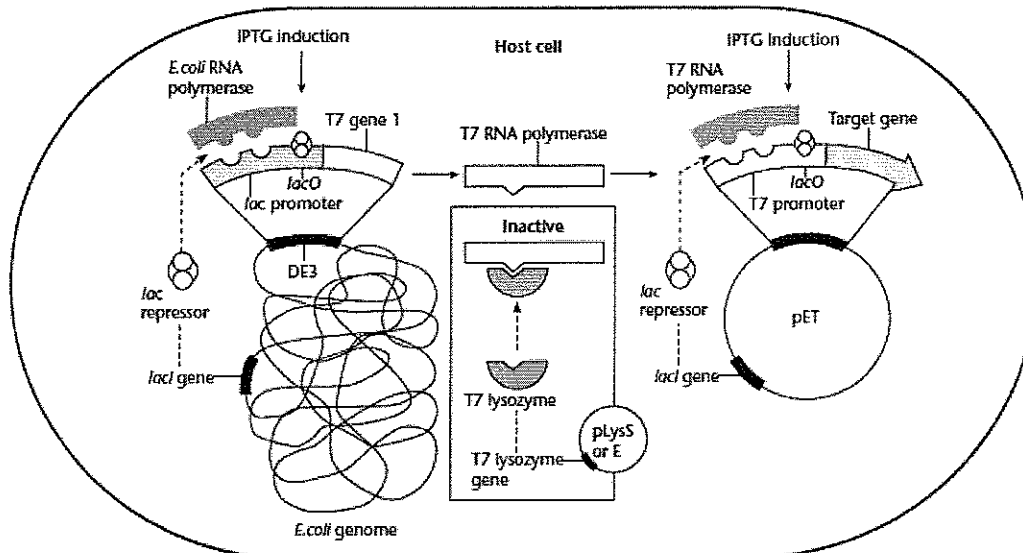
Both, pUC and the M13 *ori* regions present. Replicates as a double-stranded plasmid but, on infection with helper phage (M13KO7), singlestranded molecules are produced and packaged in phage protein.

The single-stranded molecules produced on helper phage addition have all the features necessary for DNA sequencing.

The vectors are small (<3 kb) and with a pUC *ori* have a high copy number.

(iii) Briefly comment on the diagram given below and the concept involved.

(2)



**Ans.** Diagram shows an expression vectors pET that utilize phage T7 promoters to regulate synthesis of cloned gene products. *In this diagram*

*E. coli* strains that contain gene 1 of the phage provide phage-T7 RNA polymerase. This gene is cloned downstream of the *lac* promoter for control by IPTG induction.

The T7 RNA polymerase transcribes the foreign gene in the pET plasmid.

To minimize un induced level of T7 RNA polymerase if product is toxic, a plasmid compatible with pET vectors is selected and the T7 *lysS* gene is cloned in it. In a host cell carrying a pET plasmid, the *lysS* gene will bind any residual T7 RNA polymerase .

If a *lac* operator is placed between T7 promoter and cloned gene, this will further reduce transcription of the insert in the absence of IPTG .

Q11. (i) Briefly describe structure and organization of the Ti plasmid and mechanism of gene transfer to plants . (2)

**Ans.** Ti plasmid is a circular plasmid and has 196 genes that code for 195 proteins.

The structure and organization of the Ti plasmid is simple.

In the *nopaline* plasmid there is a *single integrated segment*, while the *octopine* T-DNA comprises of *two segments*, T<sub>L</sub> (which carries the virulence genes) and T<sub>R</sub> (which carries the genes for opine synthesis).

The two segments are transferred to the plant genome independently.

The genes in the virulence region are grouped into the *operon*, which encodes the *enzymes* responsible for *mediating transduction* of T-DNA to plant cells.

Two of these genes, *virA* and *virG* are constitutively expressed at a low level.

*VirA* is a kinase that acts as a receptor for certain phenolic compounds released by the wounded plants. Eg: acetosyringone, syringaldehyde, acetovanillone.

The induction of the *vir* gene expression results in the synthesis of proteins that form the conjugative pilus through which the T-DNA is transferred to the plant cell.

*Vir B* genes: components of the pilus

*Vir D1* and *vir D2* genes: forms endonuclease for initiating DNA transfer

*virC12* and *virC2* genes: recognize and bind to the DNA sequences close to the T-DNA.

*VirE2* genes: single stranded DNA binding proteins

This complex is transferred through the pilus into the plant cells.

The *virD2* protein is proposed to protect the T-DNA against nucleases and to integrate it into the plant genome.

The nucleus of wounded plant generally gets associated with the cytosolic membrane close to the wound site.

This ensures direct transfer of the T-DNA to the nucleus without extensive exposure to the cytosol.

Once in the nucleus, the T-DNA is thought to integrate through a process of recombination using natural nicks in the chromosome.

(ii) Can *Agrobacterium* mediated gene transfer be used to transfer genes in all types of plants, if yes, Why and if no, give the reasons. How can you use the system to transfer genes in such crops? (1)

**Ans.** No, Only in dicots because of the *Vir* operon sensing the phenolics secreted by wounded plant tissue. Wounded monocot plant tissues do not produce phenolic compounds like acetosyringone to induce the *vir* genes expression, making transfer of T-DNA difficult in these. But co-cultivation of *Agrobacterium* and rice embryos in the presence of 100mM acetosyringone resulted in successful transformation.

Q12.(i) Write a Brief note on Map of Lambda chromosome. Wild type phage lambda contains several sites for Restriction endonuclease and therefore not suitable as vectors, Limiting the choice of sites for insertion of DNA. How the lambda phage is used as a vector in that case? Explain with an example. (2)

Map of lambda Chromosome

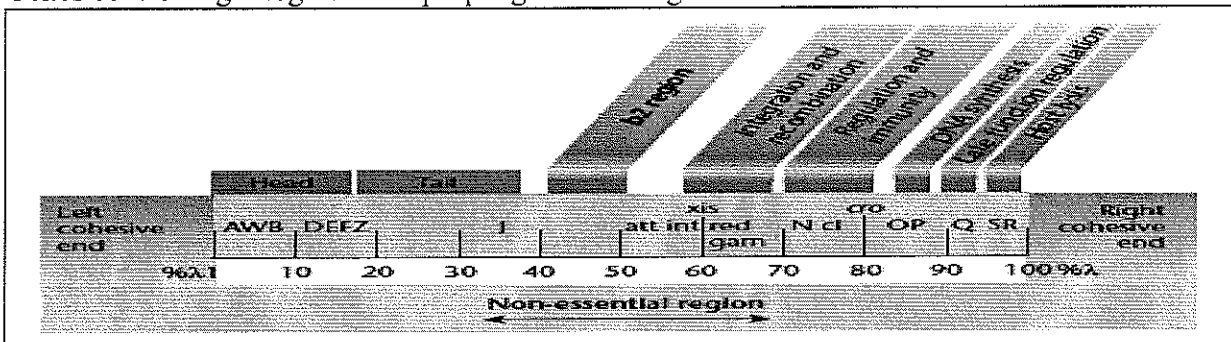
Functionally related genes (except N and Q) are clustered together

Left: head and tail proteins, Genes controlling capsid are grouped together on leftside

Central: recombination and process of lysogenization (Stuffer fragment)

Right: transcriptional regulation, immunity to superinfection, DNA synthesis, late function regulation and host cell lysis

Genes controlling integration of prophage into host genome are clustered in middle



Derivatives of phage lambda are produced by either insertion or deletion of phage genes and are being used as vectors

Phage that have a single target site at which foreign DNA can be inserted Insertional vector - DNA is inserted into a specific site

Or have a pair of sites defining a fragment that can be removed (stuffer) and replaced by foreign DNA Replacement vector - foreign DNA replaces a piece of DNA (stuffer fragment) of the vector.

A normal phage can accommodate only 5% more than its normal complement of DNA. Vector derivatives are constructed with deletions to increase the space within the genome. The shortest lambda DNA molecule that produce normal size plaques are 25% deleted.

If too much of non essential DNA is deleted from genome, it cannot be packed into phage particles efficiently

Lambda gt10 -

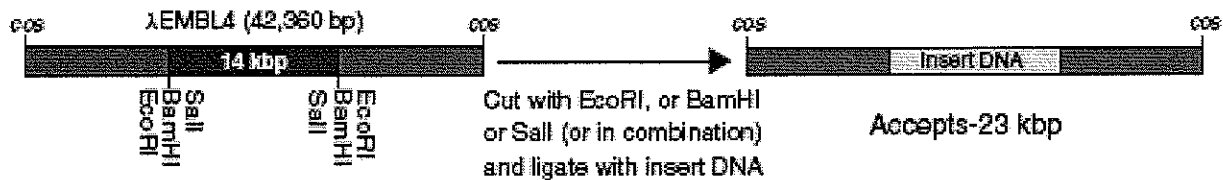
Have single Restriction site for EcoRI into which DNA can be cloned or inserted. EcoRI site lies between *cI* gene (lambda repressor) – basis for selection  
 charon 16A- site lies between lacZ gene



**Insertional**

**Accepts-10 kbp**

**Replacement (EMBL4) – has a central 13.2kb stuffer fragment flanked by inverted polylinker sequences containing sites for RE. Two SalI sites are present in stuffer fragment DNA may be inserted into any of the cloning sites**



(ii) How is the copy number of the plasmids controlled/regulated? Explain (2)

Ans. There are two mechanisms for regulation:

Anti-sense RNA (RNAI) binds to primer (RNAII)

Protein (RepA) binds to iterons

Q13. a. What is the difference between transfection and transduction? (1)

b. What are early and late function genes? Which of these is the large T antigen? (1)

c. What are the characteristic advantages of using Adeno-associated Viruses as vectors? (1)

Ans. a. Transfection- Non viral DNA transfer to eukaryotic cells, Transduction- virus-mediated DNA transfer.

b. Early genes encode enzymes and regulatory proteins needed to start viral replication processes  
 Late genes encode structural proteins, proteins needed for assembly of the mature virus (coat proteins)

Large T antigen is an early function gene.

c. ssDNA virus, naturally replication defective. In the absence of helpers, AAV DNA integrates into the host cell's genome, where it remains as a latent provirus. In human cells, the provirus integrates predominantly into the same genetic locus on chromosome 19. Subsequent infection by adenovirus or herpesvirus can "rescue" the provirus and induce lytic infection.

Advantages: Safe (because of need of helper virus), stable integration, reduced insertional mutagenesis (because of site specificity), wide host range.

**BITS PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**TEST-II (OPEN BOOK)**

**Duration: 50 min.**

**Date: 2.5.2013**

**Max. Marks: 20**

**Note:** *Answer all the questions*

*Answer to the point and draw suitable diagrams if required*

- Q1. (i) The cloning vector currently being used carry an ori derived from ColE1 and copy no. is mediated by antisense RNA, a scientist fails to understand the reason why there is a constant increase in the copy number of the plasmid, as per your understanding what do you think might be the reason for the same? Explain your answer in points. [3.0]
- (ii) You have a vector that can carry very large DNA fragments, but still there are certain problems associated with these, list those problems and describe the possible way by which these potential problems can be avoided. [3.0]
- Q2. (i) Why is it necessary to control the transgene expression precisely, what is normally done in such cases? Explain. [3.0]
- (ii) Phage promoters are preferred over bacterial promoters, Justify. [2.0]
- Q3. Eukaryotic genomic DNA libraries are important for biotechnological applications. How will you proceed for such an experiment as the genomes of eukaryotes are larger than prokaryotic genome? Explain with suitable examples and schematic diagrams. [3.0]
- Q4. For the studies on gene expression patterns genomic DNA library play a vital role. The genomic DNA clones of the human *p53* gene was isolated by screening a human genomic library in EMBL3A with a full-length cDNA probe labeled by random primer method. To synthesize cDNA, the first strand cDNA was synthesized from RNA extracted from normal human cultured cells.
- (a) Explain how you can use the cDNA for screening the genomic DNA? [2.0]
- (b) Provide schematic diagram for the strategy you follow and provide the reason for using normal human cultured cells for screening and synthesizing cDNA. [2.0]
- (c) What are the problems you may encounter and provide how you may troubleshoot to screen for the presence of *p53* gene in the genomic DNA library. [2.0]

Ans key

**BITS PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**TEST-II (OPEN BOOK)**

**Duration: 50 min.**

**Date: 2.5.2013**

**Max. Marks: 20**

**Note: Answer all the questions**

*Answer to the point and draw suitable diagrams if required*

- Q1. (i) The cloning vector currently being used carry an ori derived from ColE1 and copy no. is mediated by antisense RNA, a scientist fails to understand the reason why there is a constant increase in the copy number of the plasmid, as per your understanding what do you think might be the reason for the same? Explain your answer in points. [3.0]

Ans- (i) Absence of RNase H

(ii) Mutation in Rop Gene – Rep A protein

(iii) Temperature -42 degree.

(ii) You have a vector that can carry very large DNA fragments, but still there are certain problems associated with these, list those problems and describe the possible way by which these potential problems can be avoided. [3.0]

Ans. Ans.- BACs and PACs are used to clone large fragment of DNA (e.g. >100 kb), complete operons or very large gene clusters. However, subsequent engineering of clones is very difficult:

- Very large plasmids are prone to shearing
- They have very restricted mobility in gel electrophoresis
- Longer DNA is more likely to have multiple restriction sites. Cleavage-ligation reaction could reduce size of the DNA insert and result in scrambling of fragments.

To avoid these problems manipulations are carried out *in vivo* instead of *in vitro* using homologous recombination

- Q2. (i) Why is it necessary to control the transgene expression precisely, what is normally done in such cases? Explain. [3.0]

Ans. Because if the recombinant protein is toxic, high level would be lethal and would prevent the recovery of stably transformed cell lines.

Use of inducible expression system

(ii) Phage promoters are preferred over bacterial promoters, Justify. [2.0]

Ans. (i) They are strong, enabling large amounts of RNA to be made *in vitro*

(ii) Not recognized by E.coli RNA pol so no transcription will occur inside cell

(iii) Simpler to handle

- Q3. Eukaryotic genomic DNA libraries are important for biotechnological applications. How will you proceed for such an experiment as the genomes of eukaryotes are larger than prokaryotic genome? Explain with suitable examples and schematic diagrams. [3.0]

Genomic DNA isolation, restriction digestion, larger dna fragments, bac, yac, phage packaging.

- Q4. For the studies on gene expression patterns genomic DNA library play a vital role. The genomic DNA clones of the human *p53* gene was isolated by screening a human genomic library in EMBL3A with a full-length cDNA probe labeled by random primer method. To synthesize cDNA, the first strand cDNA was synthesized from RNA extracted from normal human cultured cells.

- (a) Explain how you can use the cDNA for screening the genomic DNA? [2.0]  
cDNA devoid of introns, oligos, labelling
- (b) Provide schematic diagram for the strategy you follow and provide the reason for using normal human cultured cells for screening and synthesizing cDNA. [2.0]  
Transcription in other systems, dna, processing.
- (c) What are the problems you may encounter and provide how you may troubleshoot to screen for the presence of *p53* gene in the genomic DNA library. [2.0]  
Digestion, separation, fragments, sequencing, probing

**BITS PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**TEST-I (CLOSED BOOK)**

**Duration: 50 min.**

**Date: 14.3.2013**

**Max. Marks: 25**

**Note:** *Answer all the questions*

*Answer to the point and draw suitable diagrams if required*

---

1. Briefly describe the immigration control region of strain *E. coli* K12, what is its significance? [4.0]
2. How isocaudamers, isoshizomers and neoschizomers are different? Explain with an example. [3.0]
3. Is it possible that a site for one restriction enzyme can be converted into a site for another RE? Explain with two examples. [4.0]
4. Briefly outline the polymerase chain reaction and mention the recent developments on minimizing the amplification times. [3.0]
5. What is RT-PCR and mention the enzymes used for cDNA synthesis? Outline the three strategies for the synthesis of first-strand cDNA synthesis. [4.0]
6. Write a short note on MDA of whole genome amplification with suitable enzymes and the principle involved. [3.0]
7. How gene library is made with the help of lambda phage DNA? Mention the principle and the method on specific use of restriction enzymes while preparing the genomic DNA for genomic DNA library. [4.0]

Ans. leung

**BITS PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**TEST-I (CLOSED BOOK)**

**Duration: 50 min.**

**Date: 14.3.2013**

**Max. Marks: 25**

**Note:** *Answer all the questions*

*Answer to the point and draw suitable diagrams if required*

1. Briefly describe the immigration control region of strain *E. coli* K12, what is its significance? [4.0]  
hsdS, seq, mutations, mcr
2. How isocaudamers, isoshizomers and neoschizomers are different? Explain with an example. [3.0]  
Based on recognition sequence and recognition
3. Is it possible that a site for one restriction enzyme can be converted into a site for another RE? Explain with two examples. [4.0]  
Yes, methylation
4. Briefly outline the polymerase chain reaction and mention the recent developments on minimizing the amplification times. [3.0]  
DNA, DNA pol, ramp rates
5. What is RT-PCR and mention the enzymes used for cDNA synthesis? Outline the three strategies for the synthesis of first-strand cDNA synthesis. [4.0]  
RNA to DNA, RNA pol, viral pol, oligos, random primer, specific
6. Write a short note on MDA of whole genome amplification with suitable enzymes and the principle involved. [3.0]  
Phi29 pol, whole genome amplification, 30 degrees, fast error free synthesis
7. How gene library is made with the help of lambda phage DNA? Mention the principle and the method on specific use of restriction enzymes while preparing the genomic DNA for genomic DNA library. [4.0]  
Methylation, internal sequences, phage packaging.

**BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**QUIZ-II (CLOSED BOOK)**

**Duration: 20 min.**

**Date: 26.5.2013**

**Weightage: 7%**

**Max. Marks: 7**

**Name:** \_\_\_\_\_

**ID No:** \_\_\_\_\_

*Note: a) answer all the questions and b) answer to the point*

1. How the secondary structure formation of DNA affect, and mention any two points to overcome the secondary structure in sequencing gel? [1.0]
  
2. Why the capillary electrophoresis is preferred in automated DNA sequencing reactions over gel electrophoresis? [1.0]
  
3. Most chemical transfection methods are based on the \_\_\_\_\_ nature of \_\_\_\_\_ (1M)
  
4. In the calcium chloride method calcium chloride\_ is added to DNA \_\_\_\_\_ buffer just before transfection because the \_\_\_\_\_ precipitate needs to be freshly formed. (1M)
  
5. A recipient cell line with non-functional \_\_\_\_\_ gene is used for transformation and selection on a \_\_\_\_\_ medium containing \_\_\_\_\_. In this case the selectable marker incorporated into the vector will be a functional \_\_\_\_\_ gene. (2M)
  
6. Replication defective viral vectors require either \_\_\_\_\_ or \_\_\_\_\_ to provide missing viral functions. (1M)

Ans. Long

**BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**QUIZ-II (CLOSED BOOK)**

**Duration: 20 min.**

**Date: 26.5.2013**

**Weightage: 7%**

**Max. Marks: 7**

**Name:**

**ID No:**

*Note: a) answer all the questions and b) answer to the point*

1. How the secondary structure formation of DNA affect, and mention any two points to overcome the secondary structure in sequencing gel? [1.0]  
Mobility in PAGE, 7M urea, temp at 70 degrees.
2. Why the capillary electrophoresis is preferred in automated DNA sequencing reactions over gel electrophoresis? [1.0]  
Longer preparation time, reproducibility, reading the gel, quantity.
3. Most chemical transfection methods are based on the negatively charged nature of plasma membrane. (1M)
4. In the calcium chloride method Calcium chloride is added to DNA in phosphate buffer just before transfection because the calcium phosphate- DNA precipitate needs to be freshly formed. (1M)
5. A recipient cell line with non-functional HPRT (hypoxanthine-guanine phosphoribosyltransferase) gene is used for transformation and selection on a HAT medium containing hypoxanthin, aminopterin and thymine. In this case the selectable marker incorporated into the vector will be a functional HPRT gene. (2M)
6. Replication defective viral vectors require either a packaging cell line or helper virus to provide missing viral functions. (1M)

**BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**QUIZ-I (CLOSED BOOK)**

**Duration: 20 min.**

**Date: 25.2.2013**

**Weightage: 8%**

**Max. Marks: 8**

**Name:**

**ID No:**

---

*Note: a) answer all the questions and b) answer to the point*

---

1. How the RFLP help identify mutations in genes? Briefly outline a method explaining the principle with a suitable diagram. [1.5]
  
2. What are microsattelites? Mention any two applications. [1.5]
  
3. What is the basic difference between the agarose gel electrophoresis and PFGE/CHEF. Mention the principle of DNA migrations and two applications for each. [2.0]
  
4. What are the two different methods by which the transfer of DNA/protein from gel is transferred to NC membranes with high efficiency? [1.0]
  
5. What is stringency washes and why this is employed during hybridization? Briefly outline two methods by which high and low stringency conditions created during hybridization. [2.0]

Ans. Cey

**BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**QUIZ-I (CLOSED BOOK)**

**Duration: 20 min.**

**Date: 25.2.2013**

**Weightage: 8%**

**Max. Marks: 8**

**Name:**

**ID No:**

*Note: a) answer all the questions and b) answer to the point*

1. How the RFLP help identify mutations in genes? Briefly outline a method with a suitable diagram. [1.5]  
Change in a base change restriction site, gel
2. What are microsatellites? Mention any two applications. [1.5]  
Small conserved sequences in DNA, used to identify the inheritance pattern, origin
3. What is the basic difference between the agarose gel electrophoresis and PFGE/CHEF. [2.0]  
Mention the principle of DNA migrations and two applications for each.  
Small size, large size, different orientation.
4. What are the two different methods by which the transfer of DNA/protein from gel is transferred to NC membranes with high efficiency? [1.0]  
Electroelution, vacuum
5. What is stringency washes and why this is employed during hybridization? Briefly outline two methods by which high and low stringency conditions created during hybridization. [2.0]  
Complementary sequences, hybridization, salt and temperature.

**BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**QUIZ-I (CLOSED BOOK)**

**Duration: 20 min.**

**Weightage: 8%**

**Date: 25.2.2013**

**Max. Marks: 8**

**Name:**

**ID No:**

---

*Note: a) answer all the questions and b) answer to the point*

---

1. How the RFLP help identify mutations in genes? Briefly outline a method explaining the principle with a suitable diagram. [1.5]
  
  
  
  
  
  
  
  
  
  
2. What are microsatellites? Mention any two applications. [1.5]
  
  
  
  
  
  
  
  
  
  
3. What is the basic difference between the agarose gel electrophoresis and PFGE/CHEF. Mention the principle of DNA migrations and two applications for each. [2.0]
  
  
  
  
  
  
  
  
  
  
4. What are the two different methods by which the transfer of DNA/protein from gel is transferred to NC membranes with high efficiency? [1.0]
  
  
  
  
  
  
  
  
  
  
5. What is stringency washes and why this is employed during hybridization? Briefly outline two methods by which high and low stringency conditions created during hybridization. [2.0]

Ans. key.

**BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS**  
**DUBAI INTERNATIONAL ACADEMIC CITY**  
**SECOND SEMESTER 2012 – 2013**  
**BIOT C461 RECOMBINANT DNA TECHNOLOGY**  
**QUIZ-I (CLOSED BOOK)**

**Duration: 20 min.**

**Date: 25.2.2013**

**Weightage: 8%**

**Max. Marks: 8**

**Name:**

**ID No:**

*Note: a) answer all the questions and b) answer to the point*

1. How the RFLP help identify mutations in genes? Briefly outline a method with a suitable diagram. [1.5]  
Change in a base change restriction site, gel
2. What are microsatellites? Mention any two applications. [1.5]  
Small conserved sequences in DNA, used to identify the inheritance pattern, origin
3. What is the basic difference between the agarose gel electrophoresis and PFGE/CHEF. [2.0]  
Mention the principle of DNA migrations and two applications for each.  
Small size, large size, different orientation.
4. What are the two different methods by which the transfer of DNA/protein from gel is transferred to NC membranes with high efficiency? [1.0]  
Electroelution, vacuum
5. What is stringency washes and why this is employed during hybridization? Briefly outline two methods by which high and low stringency conditions created during hybridization. [2.0]  
Complementary sequences, hybridization, salt and temperature.