SSR PG COLLEGE NIZAMABAD

M,Sc Biotechnology

Semester – III

PAPER – I, INTERNAL - I

r – DNA TECHNOLOGY

Credit I: Enzymes and Vectors Used in Molecular Cloning

1. The primary function of a restriction modification system in a bacterial host is to:
a) Facilitate horizontal gene transfer
b) Degrade foreign DNA while protecting its own
c) Increase the rate of DNA replication
d) Synthesize new restriction enzymes
Answer: b) Degrade foreign DNA while protecting its own

2. Which type of restriction endonuclease is most commonly used in molecular cloning due to its specific recognition sequence and cleavage site?
a) Type I
b) Type II
c) Type III
d) Type IV
Answer: b) Type II

3. The enzyme that catalyzes the formation of a phosphodiester bond between two DNA fragments is:
a) DNA Polymerase
b) Alkaline Phosphatase
c) DNA Ligase
d) Polynucleotide Kinase
Answer: c) DNA Ligase

4. The pBR322 plasmid is often used as a cloning vector. It contains genes for antibiotic resistance. Which two antibiotics are commonly used as selectable markers in pBR322?
a) Kanamycin and Tetracycline
b) Ampicillin and Kanamycin
c) Ampicillin and Tetracycline
d) Chloramphenicol and Ampicillin
Answer: c) Ampicillin and Tetracycline

5. Which feature of the pUC18 plasmid allows for blue-white screening of recombinant colonies?
a) The lacZ gene
b) The tetracycline resistance gene
c) A cos site
d) The origin of replication
Answer: a) The lacZ gene

6. An expression vector like pET21 is designed specifically for:
a) Replicating in multiple host species

b) High-level transcription and translation of a cloned gene
c) Accommodating very large DNA inserts
d) Preparing genomic libraries
Answer: b) High-level transcription and translation of a cloned gene

7. Which vector is most suitable for cloning large genomic DNA fragments (30-45 kb)?
a) Plasmid pUC18
b) Bacteriophage M13
c) Cosmid
d) Yeast Artificial Chromosome (YAC)
Answer: c) Cosmid

8. Bacteriophage λ vectors are primarily used for:
a) Protein expression in mammalian cells
b) DNA sequencing by the Sanger method
c) Construction of genomic and cDNA libraries
d) Cloning single-stranded DNA
Answer: c) Construction of genomic and cDNA libraries

9. A vector that can replicate in two different host organisms (e.g., E. coli and yeast) is called a:
a) Phagemid
b) Shuttle vector
c) Phasmid
d) Viral vector
Answer: b) Shuttle vector

10. The enzyme Reverse Transcriptase (RNA-dependent DNA polymerase) is crucial for:
a) Digesting genomic DNA
b) Synthesizing cDNA from an mRNA template
c) Phosphorylating the 5' ends of DNA
d) Methylating DNA to protect it from restriction enzymes
Answer: b) Synthesizing cDNA from an mRNA template

11. Terminal Deoxynucleotidyl Transferase (TdT) adds nucleotides to the 3' end of DNA without a template. This property is useful for:
a) Creating blunt ends
b) DNA sequencing
c) Adding homopolymeric tails in cDNA cloning
d) Restriction digestion
Answer: c) Adding homopolymeric tails in cDNA cloning

12. Alkaline Phosphatase is used in cloning to remove 5' phosphate groups. This prevents:
a) Ligation of the vector without an insert
b) Digestion by restriction enzymes
c) Transcription of the insert
d) Replication of the plasmid
Answer: a) Ligation of the vector without an insert

13. Which viral vector is commonly used for protein expression in insect cells?
a) SV40
b) Cauliflower Mosaic Virus (CaMV)

c) Baculovirus
d) M13 phage
Answer: c) Baculovirus

14. A Yeast Artificial Chromosome (YAC) is capable of cloning very large DNA inserts up to:
a) 10 kb
b) 50 kb
c) 200 kb
d) 2000 kb (2 Mb)
Answer: d) 2000 kb (2 Mb)

15. The key difference between a phagemid and a regular plasmid is that a phagemid:
a) Contains a phage origin of replication
b) Cannot replicate in E. coli
c) Lacks an antibiotic resistance gene
d) Is always single-stranded
Answer: a) Contains a phage origin of replication

16. The enzyme Polynucleotide Kinase is used to:
a) Degrade RNA from a DNA-RNA hybrid
b) Add a phosphate group to the 5' end of DNA
c) Remove phosphate groups from DNA
d) Synthesize DNA from a RNA template
Answer: b) Add a phosphate group to the 5' end of DNA

17. Which vector is derived from a plant virus and has been used in plant genetic engineering?
a) SV40
b) BAC
c) Cauliflower Mosaic Virus (CaMV)
d) λ phage
Answer: c) Cauliflower Mosaic Virus (CaMV)

18. The main advantage of a Bacterial Artificial Chromosome (BAC) over a YAC is:
a) Larger insert size capacity
b) Higher stability and easier manipulation in bacteria
c) Ability to express eukaryotic genes correctly
d) Presence of a selectable marker for blue-white screening
Answer: b) Higher stability and easier manipulation in bacteria

19. In the context of restriction enzymes, "sticky ends" are created when the enzyme:
a) Cuts both DNA strands at the same position
b) Makes a staggered cut, producing short, single-stranded overhangs
c) Requires ATP for its activity
d) Cleaves DNA at a distance from its recognition site
Answer: b) Makes a staggered cut, producing short, single-stranded overhangs

20. Methylases are enzymes that protect host DNA from its own restriction enzymes by:
a) Degrading the restriction enzyme
b) Adding methyl groups to specific bases within the recognition sequence
c) Sealing nicks in the DNA backbone

d) Unwinding the DNA double helix
Answer: b) Adding methyl groups to specific bases within the recognition sequence

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Credit II: Construction of Genomic and cDNA Libraries

1. The fundamental difference between conventional cloning and recombinant DNA-based cloning is that recombinant technology involves:
a) Cloning within the same organism
b) Joining DNA molecules from different sources in vitro
c) Using only natural plasmids
d) Cloning without the use of enzymes
Answer: b) Joining DNA molecules from different sources in vitro

2. The first step in constructing a genomic library is to:
a) Ligate the DNA into a vector
b) Transform the host bacteria
c) Isolate and fragment the genomic DNA
d) Screen for recombinant clones
Answer: c) Isolate and fragment the genomic DNA

3. For the construction of a representative genomic library, genomic DNA is often partially digested with a restriction enzyme. Why is a partial digestion preferred?
a) To generate uniformly small fragments
b) To create random fragments of a clonable size, ensuring all genomic regions are represented
c) To ensure all fragments have the same sticky ends
d) To prevent the DNA from being cut at all
Answer: b) To create random fragments of a clonable size, ensuring all genomic regions are represented

4. Chromosome walking is a technique used for:
a) Rapid DNA sequencing
b) Isolating adjacent sequences of DNA to map and clone a gene
c) Constructing cDNA libraries
d) Separating chromosomes by gel electrophoresis
Answer: b) Isolating adjacent sequences of DNA to map and clone a gene

5. Chromosome jumping libraries are particularly useful for:
a) Studying gene expression levels
b) Bypassing large regions of repetitive or unclonable DNA during positional cloning
c) Expressing proteins in bacteria
d) Cloning short cDNA fragments
Answer: b) Bypassing large regions of repetitive or unclonable DNA during positional cloning

6. The key component needed to start the construction of a cDNA library is:
a) Genomic DNA
b) Total RNA or mRNA
c) Restriction enzymes
d) DNA Ligase
Answer: b) Total RNA or mRNA

7. The enzyme essential for synthesizing the first strand of cDNA from an mRNA template is:
a) DNA-dependent DNA polymerase
b) Taq polymerase
c) Reverse transcriptase
d) RNA polymerase
Answer: c) Reverse transcriptase

8. A subtracted cDNA library is enriched for:
a) All genes expressed in a cell
b) Genes that are expressed in one cell type but not in another
c) Highly repetitive genomic sequences
d) Non-coding RNAs
Answer: b) Genes that are expressed in one cell type but not in another

9. A normalized library is treated to:
a) Increase the representation of rare transcripts
b) Reduce the representation of abundant transcripts, equalizing the frequency of all cDNAs
c) Remove all intronic sequences
d) Add specific restriction sites
Answer: b) Reduce the representation of abundant transcripts, equalizing the frequency of all cDNAs

10. What does PCR stand for?
a) Protein Copying Reaction
b) Polymerase Chain Reaction
c) Primer Controlled Replication
d) Phage Cloning Reaction
Answer: b) Polymerase Chain Reaction

11. The three main steps in a standard PCR cycle, in order, are:
a) Ligation, Transformation, Screening
b) Denaturation, Annealing, Extension
c) Digestion, Ligation, Polymerization
d) Synthesis, Degradation, Methylation
Answer: b) Denaturation, Annealing, Extension

12. The thermostable DNA polymerase commonly used in PCR was isolated from the bacterium:
a) *Escherichia coli*
b) *Thermus aquaticus* (Taq polymerase)
c) *Bacillus subtilis*
d) *Agrobacterium tumefaciens*
Answer: b) *Thermus aquaticus* (Taq polymerase)

13. Which type of PCR is used to quantify the amount of a specific DNA sequence in a sample?
a) Nested PCR
b) RT-PCR
c) Real-time PCR (qPCR)
d) Inverse PCR
Answer: c) Real-time PCR (qPCR)

14. Reverse Transcription PCR (RT-PCR) is used to amplify:
a) Genomic DNA

b) Protein sequences
c) DNA copies of RNA sequences
d) Plasmid DNA
Answer: c) DNA copies of RNA sequences

15. The purpose of the "Annealing" step in PCR is to:
a) Separate the double-stranded DNA template
b) Synthesize new DNA strands
c) Allow primers to bind to their complementary sequences on the template DNA
d) Degrade the original template
Answer: c) Allow primers to bind to their complementary sequences on the template DNA

16. Which application is NOT a common use of PCR?
a) DNA fingerprinting and forensics
b) Diagnosis of genetic diseases and infections
c) Cloning large genomic fragments directly without vectors
d) Site-directed mutagenesis
Answer: c) Cloning large genomic fragments directly without vectors

17. In a genomic library, each clone contains:
a) A copy of a specific mRNA
b) A fragment of the entire genome
c) A fully functional gene ready for expression
d) Only non-coding DNA
Answer: b) A fragment of the entire genome

18. A cDNA library represents:
a) The entire genome, including introns and exons.
b) Only the genes that are being expressed as mRNA in the cell/tissue at the time of RNA isolation.
c) All mitochondrial DNA.
d) Repetitive sequences of the genome.
Answer: b) Only the genes that are being expressed as mRNA in the cell/tissue at the time of RNA isolation.

19. What is the primary goal of "positional cloning"?
a) To determine the 3D structure of a protein
b) To clone a gene based on its map position on a chromosome, without prior knowledge of the gene product
c) To clone genes that are positionally expressed in the nucleus
d) To create a library of cDNA sequences
Answer: b) To clone a gene based on its map position on a chromosome, without prior knowledge of the gene product

20. Which technique would be most appropriate if you wanted to study only the genes that are differentially expressed in a cancer cell compared to a normal cell?
a) Construct a genomic library
b) Perform chromosome walking
c) Construct a subtracted cDNA library
d) Use Inverse PCR
Answer: c) Construct a subtracted cDNA library

SHORT ASNWER QUESTIONS

1.Define Restriction endonuclease

2.Write a short note on pBR322

3.Define Shuttle vector

4.Mention the steps involved in PCR

5.Define C-DNA Libraries