

*Virtual University of Pakistan*  
*Final term papers*  
*BIO203*  
*Method of molecular Biology*

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Bio203

Difference bw linker and adaptor

The main difference between linker and adaptor is that former having blunt ends while later have one cohesive end

Chemically synthesized adaptor molecules which have a preformed cohesive end can be used to insert blunt ended foreign DNA into vector

Adaptors

Difference bw type II And type IIs

Type II

- Most of the useful R-M system is Type II
- Type II enzymes recognize a defined sequence and cut within it

Type IIs

- Type IIs systems have similar cofactors and structure to type II but restriction occurs at a distance from recognition site that limits their usefulness

Mechanism of wga

Whole genome amplification (WGA) is a group of procedures that allow amplification to occur at many locations in a genome.

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Mechanism of DNA ligase

DNA ligase

■ An enzyme that creates a phosphodiester bond between the 3' end of one DNA segment and the 5' of another

DNA ligase

■ *E. coli* and phage T4 encode enzyme, DNA ligase, which seals single stranded nicks between adjacent nucleotides in a duplex DNA chain

i). Joining covalently annealed cohesive ends by DNA ligase

ii). Joining blunt-ended fragments by DNA ligase from phage T4 infected *E. coli*

iii). The third utilizes the enzyme terminal deoxynucleotidyltransferase to synthesize homopolymeric 3' single stranded tails at the ends of fragments

DNA ligase can repair the nicks produced after the association of cohesive ends of DNA strands

Limitations of PCR

**limitations**

- Contamination risk
- Primer complexities
- Primer-binding site complexities
- Amplifies rare species
- Detection methods

Western blotting procedure

**Procedure**

- 1- Sample preparation
- 2- Gel Electrophoresis
- 3- Blotting (or transfer)
- 4- Blocking
- 5- Antibody probing
- 6- Detection

**Procedure**

- 1- The choice of extraction method depends primarily on the sample and whether the analysis is targeting all the proteins in a cell or only a component from a particular subcellular fraction.
- 2- Samples are loaded into separate wells. A protein marker is also loaded. The separated protein mixtures are transferred to a solid support for further analysis.

3- Transfer can be done in wet or semi-dry conditions. Semi-dry transfer is generally faster. Wet transfer is recommended for large proteins, >100 kD.

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4- Blocking is a very important step in the immunodetection phase of Western blotting because it prevents non-specific binding of antibody to the blotting membrane.

Properties of DNA probe

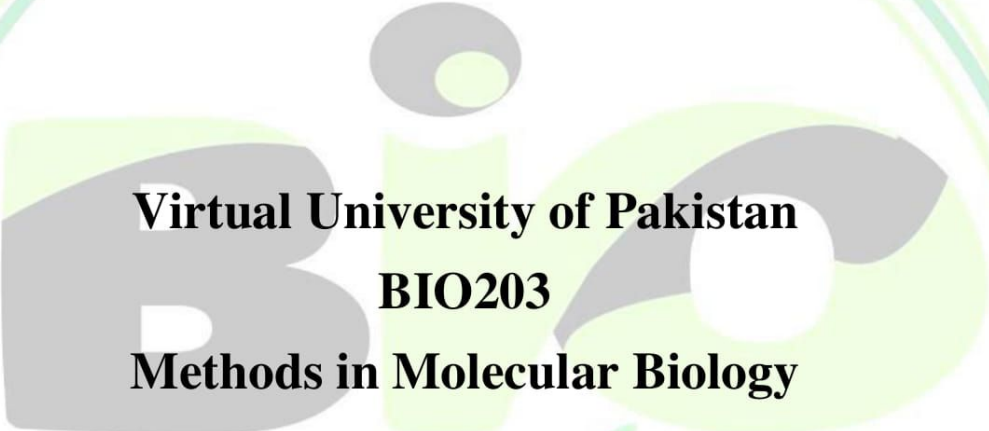
Nomenclature of restrictions enzyme. Also explain mini primer per

Calculate restriction sites every four, six, eight.



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1. Difference between linker and adaptor
2. Write difference between type II and type IIs in R-M system?
3. Mechanism of WGA
4. Mechanism of DNA ligase
5. Limitations of pcr
6. Western blotting procedure
7. Properties of DNA probe
8. Nomenclature of restrictions enzyme. Also explain mini primer pcr
9. Calculate restriction sites every four, six, eight.
10. Mini-primer PCR
11. Calculate restriction enzyme bases of 4, 6 and 8?
12. Defined the properties of probe?
13. What is the purpose of blank control and negative control in PCR reaction?
14. What is allu-PCR and write it purpose?
15. Write difference between type II and type IIs in R-M system?
16. Write down nomenclature in joining DNA molecules?
17. What is southern blot and how DNA cleaved?
18. Mutation identification?
19. Use of assmyteric PCR?
20. What is the first step of RT.PCR
21. What is the use of methylation PCR
22. Whrite a note on mini PCR
23. Why we use Sourthern blotting technique
24. Inverse PCR and conventional PCR difference
25. What solid support is used in southern blotting and hybridization components?
26. Blank and positive control reaction .

27. Pfu,taq and vent source and extension rate.
28. Primer designing for whole genome amplification PCR.
29. Limitations of PCR.
30. Southern blotting principle
31. Nomenclature of enzymes
32. Mini primer PCR
33. Southern blotting probe.
34. DNA ligase mechanism.
35. Properties of DNA probe

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Q=Difference bw linker and adaptor

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