



# KENYATTA UNIVERSITY

## UNIVERSITY EXAMINATIONS 2011/2012

### SECOND SEMESTER EXAMINATION FOR THE DEGREE OF BACHELOR OF SCIENCE IN BIOCHEMISTRY AND BIOTECHNOLOGY

#### SBC 316: ADVANCED LABORATORY TECHNIQUES

DATE: Monday 26<sup>th</sup> March 2012

TIME: 2.00p.m- 4.00p.m

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**INSTRUCTIONS: Section A: Answer ALL Questions; Choose the Best Answer(1 mark each)**

- 1) The resolution of a chromatographic separation can be increased by all of the following except:
  - A) A longer column
  - B) A stronger mobile phase
  - C) A more efficient column
  - D) A stationary phase with higher compound retention
  
- 2) All the following are characteristic of HPLC except:
  - A) Small particle matrices
  - B) Fast separations
  - C) Difficult to automate
  - D) Narrow peaks
  
- 3) Which of the following ion – exchange media could not be used effectively at pH 5?
  - A) Strong cation exchanger
  - B) Weak cation exchanger
  - C) Strong anion exchanger
  - D) Weak anion exchanger

- 4) Which of the following electrophoretic media show molecular sieving effects?
- A) Cellulose acetate membranes
  - B) Agarose gel
  - C) Polyacrylamide gel
  - D) Starch gel
- 5) SDS – PAGE is most often used to:
- A) Purify native proteins
  - B) Evaluate molecular weight of proteins
  - C) Separate glycoproteins
  - D) Purify denatured proteins
- 6) Normal phase chromatography is most often used to purify:
- A) Positively - charged molecules
  - B) Negatively – charged molecules
  - C) Non – polar molecules.
  - D) Polar molecules
- 7) Reverse-phase chromatography is most often used to purify:
- A) Hydrophobic molecules
  - B) Hydrophilic molecules
  - C) Positively – charged molecules
  - D) Negatively – charged molecules
- 8) Lectin-affinity chromatography is most often used to purify:
- A) Hydrophobic molecules
  - B) Negatively- charged - molecules
  - C) Positively-charged molecules
  - D) Glycoproteins
- 9) A chromatographic method that involves specific but reversible interaction between immobilized ligands and biological molecules in the sample is called:
- A) Ion-pair chromatography
  - B) Size – exclusion chromatography
  - C) Affinity chromatography
  - D) Ion-exchange chromatography

10) Ampholytes or ampholines are used in what method?

- A) SDS-PAGE
- B) Agarose gel electrophoresis
- C) Discontinuous-pH PAGE
- D) Isoelectric focussing

11) Genetic engineering involves which of the following?

- A) Conjugation
- B) Derivatization
- C) Cloning
- D) Mutation

12) DNA Cloning requires the following except:

- A) A vector
- B) Restriction endonucleases
- C) DNA ligase
- D) Methylase

13) DNA containing a genetic sequence that is to be cloned is referred to:

- A) Vector DNA
- B) Donor DNA
- C) Single-stranded DNA
- D) Host DNA

14) For extraction of DNA from cells, all of the following is required except:

- A) Detergent
- B) Organic solvents
- C) Potassium salts
- D) Ethanol

15) Bacterial enzymes that cut DNA at specific sites within DNA molecules are called:

- A) Exonuclease
- B) Methylase
- C) Transferases
- D) Restriction endonucleases

16) The site at which restriction enzyme cuts DNA is called:

- A) Palindromic DNA
- B) Recognition sequences
- C) Sticky ends
- D) Blunt ends

17) Restricted DNA can be separated using the following technique:

- A) HPLC
- B) Electrophoresis
- C) Centrifugation
- D) Restriction mapping

18) All the following are descriptive of cloning vectors except:

- A) Plasmids
- B) Bacteriophages
- C) Genomic DNA
- D) Shuttle

19) Which of the following enzymes splice together two pieces of DNA that has been cut by the same restriction enzymes?

- A) Kinase
- B) Ligase
- C) Polymerase
- D) Lyase

20) All the following are common methods for experimentally introducing DNA into host cells except:

- A) Transformation
- B) Transduction
- C) Conjugation
- D) Electroporation

**Section B: Answer ALL Questions (5 marks each)**

- 1) List the main factors governing the choice of matrix for affinity chromatography.
- 2) Explain the procedure of eukaryotic mRNA purification by chromatography.
- 3) Outline the principle of protein purification by gel filtration chromatography.
- 4) Explain the principle of DNA separation by agarose gel chromatography.
- 5) Fill in the blanks

Use of antibodies as the i)----- is exploited in the isolation and purification of ii)----- . Antibodies are covalently linked to iii) ----- using iv) ----- . Proteins binding to immobilized antibodies is achieved using v) ----- -- containing vi) ----- . Elution of vii) often requires harsh conditions because of the tight binding of viii) ----- to ix) ----- and this may lead to x) ----- .

- 6) Fill in the blanks

i) ----- is the most efficient and rapid technique for ii) ----- -- purification. However there are many iii) ----- for which iv) ----- are not easily immobilized on chromatographic matrices. The use of v) ----- has made it possible to purify any vi) ----- by vii) ----- . Initially, the gene encoding the desired protein that binds to a ligand with high affinity and specificity is prepared. The protein used for this purpose may be attached to either viii) ----- or ix) ----- is called x) -----

**Section C: Answer any TWO Questions (10 marks each)**

- 1) Describe the principle of protein purification using two-dimensional polyacrylamide gel chromatography.
- 2) Using a flowchart, outline the major steps involved in gene cloning.
- 3) Explain the principle of ion-exchange chromatography.