UNIVERSITY OF JOHANNESBURG

FACULTY OF SCIENCE

DEPARTMENT OF BIOTECHNOLOGY

NATIONAL DIPLOMA: BIOTECHNOLOGY

MODULE BIC2AAB

ANALYTICAL BIOCHEMISTRY 3

CAMPUS **DFC**

2018 JUNE EXAMINATION

DATE: JUNE 2018 **SESSION: 8:30 – 11:30**

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MODERATOR **PROF. J. SHAI**

DURATION: 3 HOURS **MARKS: 180**

NUMBER OF PAGES: 5 PAGES (EXCLUDING THIS ONE)

INSTRUCTIONS: CALCULATORS ARE PERMITTED (ONLY ONE PER STUDENT)

REQUIREMENTS: 1 ANSWER SCRIPT PER STUDENT

QUESTION 1

(a) Give a full and complete definition of each of the following:

(i)	Recombinant DNA	(2)
(ii)	Biotechnology	(2)
(iii)	SDS-PAGE	(2)
(iv)	Cloning vector	(2)
(v)	Transformation (with reference to recombinant DNA technology)	(2)
(vi)	Polymerase chain reaction	(2)
(vii)	Agarose Gel electrophoresis	(2)
(viii)	Sanger dideoxy sequencing method	(2)
(ix)	Nanobiotechnology	(2)
(x)	Nanoparticle	(2)

Consider figure 1 shown below and answer the questions that follow.

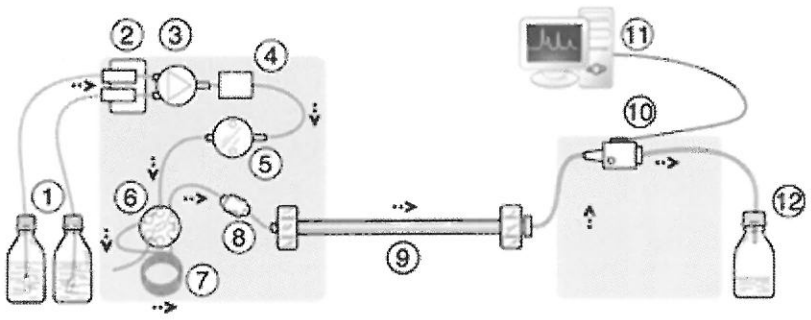

Figure 1: Schematic diagram of an HPLC system.

(b) Identify the components carrying or responsible for the following:

Mobile phases, generation of a gradient and detection. (3)

(c) What are the two possible stationery phases that may be contained in component 9 (2)

(d) Describe the major differences (in point form in terms of **method used, analytes separated, mobile phase, detectors and applications**) between GC and HPLC. (15)

(e) What would happen to the analysis process if component 9 was missing? (2)

(f) Explain in detail how component 9 works in the analysis of samples (polar and non-polar) with reference to each of the stationery phases described in (c) (6)

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QUESTION 2

(a) For each of these methods of separating proteins, describe the principle of the method, and describe the property that allows their separation by this technique.

(i) ion-exchange chromatography (4)

(ii) size-exclusion (gel filtration) chromatography (4)

(iii) affinity chromatography (4)

You have a crude lysate sample (CL) containing a mixture of six proteins (1, 2, 3, 4, 5, β-galactosidase), and your goal is to obtain purified β-galactosidase. Some characteristics of these proteins are shown in the table below

Protein	Concentration of ammonium sulphate (AS) required for precipitation	Molecular Weight (kDa)	Isoelectric point (pI)
1	45%	38	3.7
2	80%	22	4.8
3	65%	4	5.3
4	20%	75	6.8
5	30%	55	9.5
β-galactosidase	45%	115	5.3

You begin your purification by performing an ammonium sulfate (AS) precipitation. You add the appropriate concentration of AS to your CL sample, incubate overnight at 4°C, then centrifuge to generate a supernatant (AS-S) and pellet (AS-P).

(b) What concentration of AS will you use to precipitate β-galactosidase? (1)

(c) How would you best remove the AS in (b)? (1)

One way to purify β-galactosidase away from any contaminating proteins in the AS-P sample would be to separate them based on their molecular weight.

(d) What type of column separates on this basis? (1)

(e) Which protein (from your AS-P) would elute first from this type of column? (1)

Instead, you decide to use ion exchange chromatography to further purify β-galactosidase away from other proteins in your AS-P sample. You first run an anion exchange column equilibrated using column buffer with a pH of 5.0.

(f) What charge does the matrix or the resin of an anion exchange column have? (1)

(g) At pH 5.0, which protein(s) from the AS-P stick to the anion exchange column? (1)

(h) Explain your answer to part (g) in one or two sentences. (5)

(i) State how you would elute a protein bound to an anion exchange column, and explain how this elution method works in one or two sentences. (4)

You identify the fraction containing β-gal from your anion exchange column, and decide to run it over a cation exchange column to complete your purification.

(j) Describe how you would use a cation exchange column to purify β-galactosidase away from any remaining contaminating protein(s). Be specific about: (1) the pH at which you would equilibrate the column; (2) why you chose this pH; and (3) which protein(s) would bind and which protein(s) would flow through the column under these conditions, and why. (6)

Finally, you decide to run an SDS-PAGE gel to analyze your purification. You prepare your samples for loading as in lab, run your crude lysate (CL) sample (containing proteins 1-5 and β-galactosidase) on the gel, stain with Coomassie, and observe 6 bands.

(k) Which protein will migrate closest to the dye front of the SDS gel? (1)

(l) Explain your answer to (j) in one or two sentences. (4)

You analyze a second sample of CL by SDS-PAGE, but this time, you do not add β-mercaptoethanol (β-ME) to your sample buffer. You find that protein 2 has the same relative mobility in both the presence and absence of β-ME.

(m) What do these results allow you to conclude about protein 2? Justify your answer in one or two sentences. (4)

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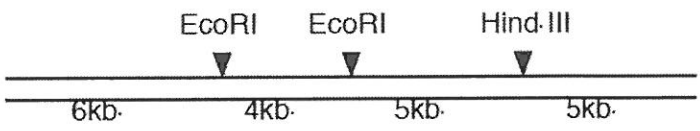
QUESTION 3

(a) After DNA fragments are separated by gel electrophoresis, how can they be visualized? (2)

(b) What is the purpose of Southern blotting? How is it carried out? (10)

(c) What are the differences between Southern, Northern, and Western blotting? (4)

You have isolated an unknown bacteriophage that has a linear genome that is 20,000 bp long and contains 2 *EcoRI* restriction sites and 1 *Hind III* site at the positions shown below. **(NOTE: FOR THIS QUESTION, RE-DRAW THE AGAROSE GEL AND LABEL TUBE A, B, C OR D ON THE WELLS)**



You isolate and purify the viral DNA and divide it into four tubes.

Tube A, you add nothing

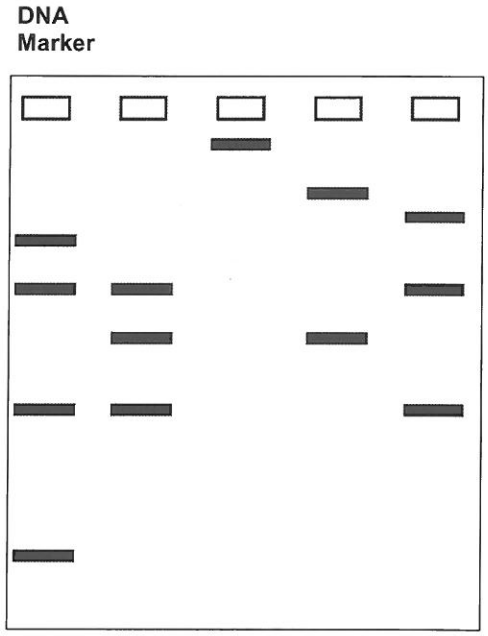
Tube B, you add *EcoRI*.

Tube C, you add *Hind III*

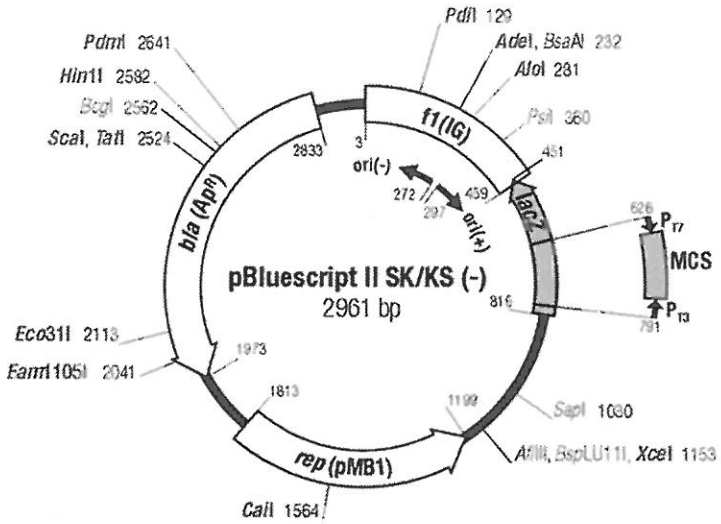
Tube D, you add both *EcoRI* and *HindIII*.

You would like to isolate the different fragments for cloning and further characterization, but as you are getting ready to load your gel, you realize that you forgot to label your tubes. Although you are extremely discouraged about your careless lab practices, you are not too worried because you have a tube with "DNA markers" that contains four fragments of known DNA sizes, a **9 kb fragment**, a **6 kb fragment**, a **4 kb fragment**, and a **2 kb fragment**. So you load your marker in the first lane and then your unlabelled samples in the following lanes of the agarose gel. Following electrophoresis, you observe the bands as shown below.

(d) On the agarose gel (next page) suggest which tube was loaded into each lane and support your answer. (10)



You work for a leading biotechnology company and one of your assignments is to isolate a cellulase gene (1000 bp) from *Bacillus subtilis* and clone it into a pBluescript (containing a *lacZ* gene) plasmid to form a pBluecellulase recombinant plasmid (pBcrp). The company has supplied you with *B. subtilis*, primers for the cellulase gene, dNTP's, DNA polymerase and *E.coli* that contain the pBluescript plasmid (shown below). **NOTE: THE MULTIPLE CLONING SITE (MCS) OF THE pBluescript contains *EcoRI*, *BamHI*, *AdeI* AND *Hin1I* restriction sites.**



(e) Describe a general procedure (in point form) you can employ to amplify the cellulase gene using some of the materials your company supplied you with. (8)

(f) How would you tell if the procedure carried out above was successful? (2)

(g) Describe the main steps you would use to isolate the pBluescript plasmid. (6)

(h) How would you verify the integrity of the isolate in (g) (that it does not contain interfering proteins). (1)

(i) After several steps, you have managed to purify the product in (e), and you now have the pBluescript plasmid. Describe how you would insert the product from (a) into the plasmid to form pBcrp. **Note: The product from (e) contains EcoRI, BamHI, Hin1I and XceI restriction sites. The last two, will cut in the middle of the gene sequence.** (4)

(j) Once the pBcrp has been transformed into competent bacterial cells, how would you select for positive transformants? Explain in POINT FORM how positive and negative transformants are generated (using pBluescript). (6)

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QUESTION 4

(a) Name and describe the different biological systems usually employed in the synthesis of nanoparticles. (6)

(b) Explain why the properties of materials can be different at the nanoscale. (6)

(c) Describe (giving examples) and explain the four categories nanoscale materials can be divided into. (8)

(d) List any 5 different applications of nanoparticles in biotechnology (5)

(e) List and briefly explain any six of the different techniques that are frequently used for the characterization of nanoparticles? (12)

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