UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biochemistry
CAMPUS	: APK
MODULE	: BICX04 CURRENT ADVANCES IN BIOTECHNOLOGY
SEMESTER	: Second
EXAM	: November 2019

DATE	: 11 November 2019	**SESSION**	: 08:30-11:30
ASSESSOR(S)	: PROF LA PIATER & Guest Lecturers		
MODERATOR	: DR L STEENKAMP		
DURATION	: 3 HOURS	**MARKS**	: 100

NUMBER OF PAGES: 5 PAGES (including this page)

INSTRUCTIONS:

1. Answer ALL THE QUESTIONS.
2. Number your answers clearly

QUESTION 1: Genes and Genomes [10]

Name and discuss, including the advantages and disadvantages, of the two strategies used for sequencing the human genome.

QUESTION 2: Yeast Biotechnology [10]

2.1 What do you understand under the term auxotrophic marker? (2)

2.2 Use an annotated, detailed diagram, or discuss, how homologous recombination can be used to introduce a desired mutation into a heterologous gene X carried in a YAC (as in the example figure below). (8)

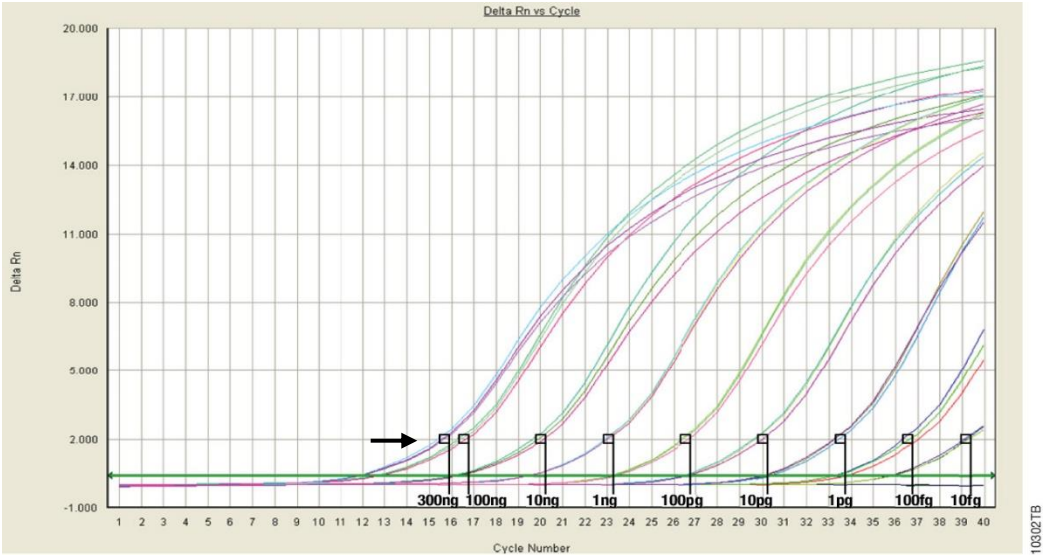
QUESTION 3: Plant Biotechnology [10]

Targeted genome editing using artificial nucleases has the potential to accelerate plant breeding by providing the means to modify genomes rapidly in a precise and predictable manner. Describe the CRISPR/Cas9 system that can be used as a gene editing method.

QUESTION 4: Molecular Diagnostics [10]

4.1. Name two different types of real-time PCR quantification methods and explain the difference between the two. (4)

4.2. With regards to real-time qPCR, what does the figure below represent? (2)



C_t

4.3 What does the "Ct" on the graph represent? (3)

4.4 Based on the concentrations shown on the graph, what was the users most likely reason for conduction this qPCR run? (1)

QUESTION 5: Biotechnology-based Drug Discovery [10]

5.1. State the drug discovery process and how long it takes on average to generate leads. (6 x ½)

5.2 Considering a genomics approach to drug discovery, explain its usefulness and state the limitations. (3)

5.3 Why are the majority of drug targets proteins? (3)

5.4 Mass spectrometry is an important technology in which of the general "omics" approaches? (1)

QUESTION 6: Vaccines [10]

List 5 features of effective vaccines, and briefly elaborate on each point.

QUESTION 7: Stem Cells **[10]**

Name the four types of (stem)-cell variants and provide advantages and disadvantages to their use in regenerative medicine purposes.

QUESTION 8: Biofuels **[10]**

8.1. Biofuels are environmentally friendly and the use of biofuels date back to 1897, where Rudolf Ford ran a diesel engine on vegetable oils.

8.1.1 Explain what is meant by environmentally friendly. (1)

8.1.2 Fuel is a material storing potential energy in form that can be released and used as heat energy. Using Bioethanol as example give the balanced chemical reaction. (1)

8.2. Name and define the two categories of biofuels. (4)

8.3 Briefly, explain how cellulosic ethanol is made. (4)

QUESTION 9: Crystallography **[15]**

9.1 In your crystallography practical in July 2019, you attempted purification of two protein components that together were meant to crystallize.

9.1.1 The target protein MAGEB1 has a molecular weight of 29 kDa, and the nanobody has 13 kDa. After purification, you have 120uL of MAGEB1 at a concentration of 3 mg/mL, and 300 uL of the nanobody at 5 mg/mL. What volumes of each solution must you mix together to yield an equimolar solution? Show your calculations. (2)

9.1.2 During purification, you first used IMAC to extract your proteins from the crude extract, and then reverse IMAC to remove remaining impurities that had also stuck to the beads during the first step. If the impurities did not have an engineered poly-histidine tail, why did they

stick to the IMAC beads in the first step, and why does reverse IMAC help remove them? (3)

9.2 Describe some advantages and disadvantages of using X-ray crystallography to elucidate the 3D structure of a protein. (2)

9.3 Discuss how protein binders such as nanobodies can act as crystallization chaperones, and increase the likelihood of a target crystallizing. (2)

9.4 Discuss in entropic terms why placing a protein in a solvent like chloroform will cause it to unfold. (3)

9.5 Why does raising the protein concentration tend to yield larger protein crystals in vapour diffusion experiments? Sketch a schematic phase diagram if it simplifies your answer. (3)

9.6 BONUS: estimate how many carbon atoms you would need to form a linear molecule long enough to transverse a bacterial cell. Describe your reasoning. (2)

QUESTION 10: Application of knowledge in project **[5]**

During your individual BICX00 research project, you made use of “omics” (genomics, proteomic, metabolomics, *etc*.) method(s) or aspects thereof (genetics using qPCR, *etc.*). Suggest an alternative approach (one not used in your project) to answer your scientific research question, and briefly give an outline of the experimental design you will follow.