UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biochemistry
CAMPUS	: APK
MODULE	: BICX04 CURRENT ADVANCES IN BIOTECHNOLOGY
SEMESTER	: Second
EXAM	: Nov 2021

DATE	: 5 November 2021	**SESSION**	: 08:30-12:30
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: Yeast Biotechnology [10]

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

1.2 Use an annotated, detailed diagram, or discuss in words, 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)

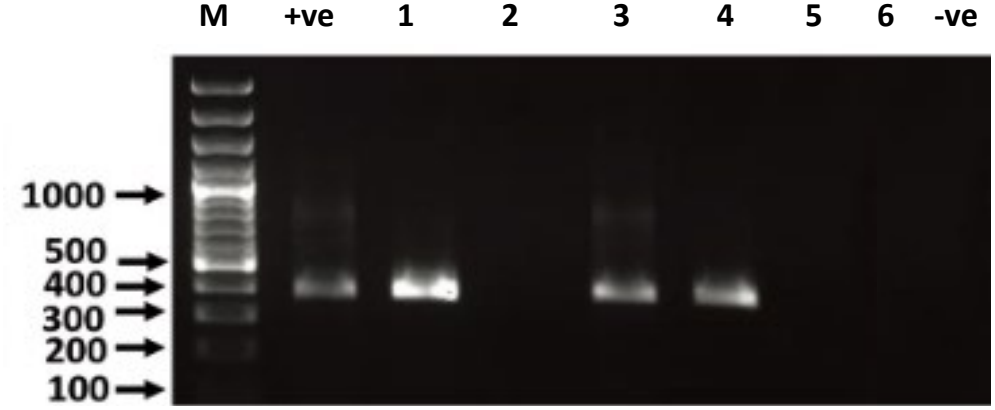
QUESTION 2: Molecular Diagnostics [15]

2.1 Describe how SYBR green is used in real-time PCR experiments to determine the amount of a target DNA gene in an unknown sample. You should include (a) how SYBR green works and (b) how SYBR green is "observed" in the data obtained by real-time PCR results. (5)

2.2 You're a plant pathologist currently working with tomato crops. A farmer in the Lanseria area notices that his tomato crops have symptoms similar to that of bacterial wilt disease which is caused by the soil-borne bacterium named *Ralstonia solanacearum*. He sends you a few samples of these symptomatic potatoes and asks you to test them for *Ralstonia solanacearum*.

2.2.1 How would go about confirming if the tomatoes where indeed infected with *Ralstonia*? (4)

2.2.2 After conducting your experiment, you get the following results. What can you conclude from these results? (4)



2.2.3 How could you further confirm that the samples are infected with *Ralstonia*? (2)

QUESTION 3: Biofuels **[15]**

3.1 Distinguish between the wet and drying milling processes. (10)

3.2 How could the wet milling process be improved? (5)

QUESTION 4: Plant Biotechnology **[15]**

There are three new plant transformation / genome editing systems on the market that can be used to speeds up trait introduction into crop species.

4.1 Name the three methods and briefly explain the advantage of using these new techniques for plant genetic transformation. (5)

4.2 These new plant transformation technologies can be used to engineer resistance to plant viruses in crop species. Name two ways that a genome editing technique that can be used to accomplish plant viral resistance in a target crop. (2)

4.3 The African cassava mosaic (ACMV) virus is a DNA-based geminivirus that infects cassava (*Manihot esculenta*) and decimates cassava production. Explain how you could use one of the above mentioned plant genome editing techniques **(that use a nucleic acid targeting system for plant transformation)** to generate a cassava line resistant to this virus. Hint: ACMV replicated within the nucleus of the cells. Make sure you explain the different components of the plant transformation/editing system and how they would work to accomplish viral resistance in the target crop. (6)

4.4 In terms of the constant arms race between pest and host, explain how the virus may evolve to evade this CRISPR-mediated defense method and how you could delay the development of such resistance in your genetically modified cassava plants. (2)

QUESTION 5: Biotechnology-based Drug Discovery **[15]**

5.1 The binding energy of a drug to a protein is given by the change in Gibbs Free Energy, which has an enthalpic and an entropic contribution:
$\Delta G = \Delta H - T\Delta S$
Name any 2 types of protein-drug interaction, and state whether they are enthalphic or entropic. (3)

5.2 For assessing a drug's ADME properties, logP measures the partition coefficient and logD the distribution constant, with $logD = logP - | 7.4 - pK_a |$

5.2.1 What behaviour of the drug does the partition coefficient quantify? (1)

5.2.2 How it is measured? (1)

5.2.3 Given the equation above, which of the two quantities (logP or logD) is affected by the charge state of the molecule, and why? (1)

5.3 Once a drug is absorbed in the small intestine, it is carried straight to the liver by the hepatic portal vein. What is the role of the liver in determining drug bioavailability, what are some of the general mechanisms it employs to affect this, and what other organ works in tandem with the liver to eliminate drugs? (3)

5.4 Given what you know about the process of drug discovery, discuss why it has been broadly assumed that it was not possible to develop a novel antiviral in time to affect the COVID-19 pandemic. (2)

5.5 Discuss exactly why an effective antiviral drug would help quell the ongoing pandemic, and why it might be more effective than a vaccine. (4)

QUESTION 6: Vaccines **[15]**

6.1 What is vaccination? Describe and distinguish between the two types of vaccinations, and give an example of each. (5)

6.2 Describe how a vaccine works. (5)

6.3 What is herd immunity and why is it important? (5)

QUESTION 7: Stem Cells and Transgenesis **[15]**

7.1 In the tissue engineering process, what are the advantages of using somatic stem cells to treat diseases? (3)

7.2 Artificial extracellular matrices frequently make use of synthetic polymers – what are three qualities that any chosen polymer should possess? (3)

7.3 Define metaplasia. (2)

7.4 Explain the process of electrospinning. (3)

7.5 Discuss the process of generating transgenic mice by DNA pronuclear injection. (4)

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QUESTION 1: Yeast Biotechnology [10]

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

Refer to slide 5 of lecture 6

1.2 Use an annotated, detailed diagram, or discuss in words, 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)

Refer to slide 10 of lecture 6

QUESTION 2: Molecular Diagnostics [15]

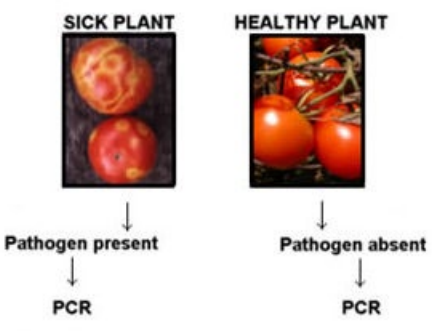
2.1 Describe how SYBR green is used in real-time PCR experiments to determine the amount of a target DNA gene in an unknown sample. You should include (a) how SYBR green works and (b) how SYBR green is "observed" in the data obtained by real-time PCR results. (5)

- SYBR green is an intercalating dye that shows increased florescence when it binds double stranded DNA.

Elaborate on fluorescence signal, quantitation of each amplification cyle and threshold cycle

2.2 You're a plant pathologist currently working with tomato crops. A farmer in the Lanseria area notices that his tomato crops have symptoms similar to that of bacterial wilt disease which is caused by the soil-borne bacterium named *Ralstonia solanacearum*. He sends you a few samples of these symptomatic potatoes and asks you to test them for *Ralstonia solanacearum*.

2.2.1 How would go about confirming if the tomatoes where indeed infected with *Ralstonia*? (4)



- Elaborate on extraction protocol, specific primer design for Genus, PCR with controls and viewing of products.

2.2.2 After conducting your experiment, you get the following results. What can you conclude from these results? (4)

Elaborate on the results with respect to the control

2.2.3 How could you further confirm that the samples are infected with *Ralstonia*? (2)

Sequencing

QUESTION 3: Biofuels **[15]**

3.1 Distinguish between the wet and drying milling processes. (10)

Refer to slides 10-12 of Biofuels lecture

3.2 How could the wet milling process be improved? (5)

Refer to slide 12 of Biofuels lecture and include insight into process

QUESTION 4: Plant Biotechnology **[15]**

There are three new plant transformation / genome editing systems on the market that can be used to speeds up trait introduction into crop species.

4.1 Name the three methods and briefly explain the advantage of using these new techniques for plant genetic transformation. (5)

Refer to slides 13 onwards of Plant Biotech lecture

4.2 These new plant transformation technologies can be used to engineer resistance to plant viruses in crop species. Name two ways that a genome editing technique that can be used to accomplish plant viral resistance in a target crop. (2)

Refer to slides 32-33 of Plant Biotech lecture

4.3 Explain how you could use one of the above mentioned plant genome editing techniques **(that use a nucleic acid targeting system for plant**

transformation) to generate a cassava line resistant to this virus. Hint: ACMV replicated within the nucleus of the cells. Make sure you explain the different components of the plant transformation/editing system and how they would work to accomplish viral resistance in the target crop. (6)

Refer to slides 41-43 of Plant Biotech lecture

4.4 In terms of the constant arms race between pest and host, explain how the virus may evolve to evade this CRISPR-mediated defense method and how you could delay the development of such resistance in your genetically modified cassava plants. (2)

Refer to slides 41-43 of Plant Biotech lecture

QUESTION 5: Biotechnology-based Drug Discovery [15]

5.1. The binding energy of a drug to a protein is given by the change in Gibbs Free Energy, which has an enthalpic and an entropic contribution:

$\Delta G = \Delta H - T\Delta S$

Name any 2 types of protein-drug interaction, and state whether they are enthalpic or entropic. (3)

Any two of the titles of slides 55-61, along with correct assignment of ΔH and ΔS.

5.2. For assessing a drug's ADME properties, logP measures the partition coefficient and logD the distribution constant, with $\log D = \log P - | 7.4 - pK_a |$

5.2.1 What behaviour of the drug does the partition coefficient quantify? (1)

Refer to slide 67 of Drug lecute

5.2.2 How it is measured? (1)

Refer to slide 67 of Drug lecture

5.2.3 Given the equation above, which of the two quantities (logP or logD) is affected by the charge state of the molecule, and why? (1)

Refer to slides 27-30 of Drug lecture

5.3. Once a drug is absorbed in the small intestine, it is carried straight to the liver by the hepatic portal vein. What is the role of the liver in determining drug bioavailability, what are some of the general mechanisms it employs to affect this, and what other organ works in tandem with the liver to eliminate drugs? (3)

Refer to slides 68-70 of Drug lecture

5.4. Given what you know about the process of drug discovery, discuss why it has been broadly assumed that it was not possible to develop a novel antiviral in time to affect the COVID-19 pandemic. (2)

(Assess whether the answer makes sense. Things they might invoke: the various stages on slide 4; that moving from each stage to the next takes time; legal barriers; paying for all of it; complexity of clinical trials. Don't hesitate to give full marks if the answer is knowledgeable.)

5.5. Discuss exactly why an effective antiviral drug would help quell the ongoing pandemic, and why it might be more effective than a vaccine. (4)

Deliberately open question – give points for well-argued answers, full marks if it's knowledgeable.

QUESTION 6: Vaccines **[15]**

6.1 What is vaccination? Describe and distinguish between the two types of vaccinations, and give an example of each. (5)

Refer to slides 2-5 for Vaccine lecture

6.2 Describe how a vaccine works. (5)

Refer to slides 5-7 for Vaccine lecture

6.3 What is herd immunity and why is it important? (5)

Refer to slide 11 for Vaccine lecture

QUESTION 7: Stem Cells and Transgenesis [15]

7.1 In the tissue engineering process, what are the advantages of using somatic stem cells to treat diseases? (3)

Refer to slide 7 for Stem cell lecture

7.2 Artificial extracellular matrices frequently make use of synthetic polymers – what are three qualities that any chosen polymer should possess? (3)

Refer to slide 16 and 17 for Stem cell lecture

7.3 Define metaplasia. (2)

Refer to slide 10 for Stem cell lecture

7.4 Explain the process of electrospinning. (3)

Refer to slides 20-22 for Stem cell lecture

7.5 Discuss the process of generating transgenic mice by DNA pronuclear injection. (4)

Refer to slides 26-28 for Stem cell lecture