UNIVERSITY OF JOHANNESBURG **FACULTY OF SCIENCE**

BOTANY APK CAMPUS
BOT3A10 PLANT BIOTECHNOLOGY

28 MAY 2018

EXAMINER **Dr E Venter**

MODERATOR **Dr NA van der Merwe**

TIME 3 HOURS **MARKS 100**

Please read the following instructions carefully
Please answer the paper using a blue pen.

This paper consists of 2 pages.

QUESTION 1 [34]

1.1 What are the implications of gene expression on plant transformation? (10)

1.2 Scientists want to study the plants that grow on another planet after successfully establishing a colony there. The atmosphere on this planet is very different to earth and the plants would possibly not grow here. Several plants produce very novel proteins for use on earth. However, it would be too costly to produce on the new planet and ship to earth. Therefore, the scientists want to use genetic engineering to transfer these genes into tobacco for growing on earth.

1.2.1. The choice of which plant can be used as a model to study the regulation and expression of the novel proteins rest on two possible candidates. Both these plants are similar in how fast they complete a lifecycle, but plant A is larger than plant B. Plant B produces a fruit with four seeds from one flower and plant A produces large amounts of seed from one flower. Which one would you choose as a model species and why would you make this choice based on the requirements of a model species. (6)

1.2.2. After sequencing the chosen model plant's genome the scientists confirm that it contains the same four nucleotides (G, A, T, C) as found for living organisms on earth and that the plant's genes do not contain introns. A difference is that the translation to amino acids is based on a four nucleotide codon rather than the triplet codon used on earth. The same amino acids used on earth are still produced in the plants, there are just different codons coding for each amino acid. As the leading scientist on earth what changes to the gene sequences would you propose so that the genes will be expressed correctly in tobacco. (4)

1.3 Explain the process of translation. (14)

QUESTION 2 [31]

2.1 Name the enzymes used in 454 sequencing. (5)

2.2 Why is a modified nucleotide used in the place of one of the dNTPs for 454 sequencing? Which nucleotide is used? (3)

2.3 Why are nucleotides added sequentially (one following the other) during most of the next generation sequencing techniques? (2)

2.4 You want to amplify a specific gene using PCR. You consult a published journal article that indicated the primer sites as underlined regions on the gene sequence (figure below). You order the primers (table below) and work out your PCR conditions. However, the first PCR that you do fails and you consult the article again to troubleshoot the problem.

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1   AGGACAGGTA CGGCTGTCAT CACTTAGACC TCACCCTGTG GAGCCACACC CTAGGGTTGG
61  CCAATCTACT CCCAGGAGCA GGGAGGGCAG GAGCCAGGGC TGGGCATAAA AGTCAGGGCA
121 GAGCCATCTA TTGCTTACAT TTGCTTCTGA CACAACTGTG TTCACTAGCA ACCTCAAACA
181 GACACCATGG TGCATCTGAC TCCTGCGGAG AAGTCTGCCG TTACTGCCCT GTGGGGCAAG
241 GTGAACGTGG ATGAAGTTGG TGGTGAGGCC CTGGGCAGGT TGGTATCAAG GTTACAAGAC
301 AGGTTTAAGG AGACCAATAG AAACTGGGCA TGTGGAGACA GAGAAGACTC TTGGGTTTCT
361 GATAGGCACT GACTCTCTCT GCCTATTGGT CTATTTTCCC ACCCTTAGGC TGCTGGTGGT
421 CTACCCTTGG ACCCAGAGGT TCTTTGAGTC CTTTGGGGAT CTGTCCACTC CTGATGCTGT
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Primers	PCR-F : TACATTTGCTTCTGACACAAC PCR-R : AACTGGGCATGTGGAGACAGAG

2.4.1 Based on the above figure, why do you think did the PCR not work, and how will you fix this problem? (2)

2.4.2 Work out the melting temperatures of both primers and indicate the temperature that you will use in your PCR for the primers to anneal to the DNA template. Your PCR protocol indicates that you are adding 2.5 mM salt in the PCR reaction. (3)

2.5 After your return from a recent trip to South America where Zika fever cases have been reported, you suffer from similar symptoms. When you visit your general practitioner he wants to find out if you were infected with the Zika virus during the previous months. Describe a simple test that he can perform on a blood sample taken from you to determine if you have contracted the Zika virus. (8)

2.6 The medicinal plant you work with has a genome size of ~2.2 x 10^6 kb. Assuming that you will clone fragments of ~8 kb in length, how many independent clones must be prepared and screened in order to have a 99% chance of including the desired clone? (8)

QUESTION 3 **[35]**

3.1 How would you design a bioreactor to ensure that relatively fast growing plant cells grown in it obtain maximum aeration and minimum shear stress? Motivate your choice and elaborate on the characteristics of the design that you choose. (14)

3.2 What are the different classes of plant growth regulators? Which ones are used to stimulate the developments of roots and shoots in tissue culture? Which ratios are used to differentiate these organs? (8)

3.3 How do organogenesis and somatic embryogenesis differ, explain fully? (13)