UNIVERSITY OF JOHANNESBURG

FACULTY OF SCIENCE

DEPARTMENT OF BIOTECHNOLOGY
BTECH: BIOTECHNOLOGY and BIOMEDICAL TECHNOLOGY

MODULE RECOMBINANT DNA TECHNOLOGY 4 BTN1YD4
MOLECULAR BIOLOGY 4 MCB41-1
CAMPUS **DFC**

NOVEMBER EXAMINATION
SECTION 2

DATE: 12/11/2012 **SESSION: 8:30 – 11:30**

ASSESSOR(S) **MR L ALAGIOZOGLOU**

EXTERNAL MODERATOR **DR K. KONDIAH**

DURATION 3 HOURS **MARKS FOR SECTION 2 90**

FULL MARKS 150

NUMBER OF PAGES: 5 PAGES

INSTRUCTIONS: THIS ASSESSMENT IS COMPOSED OF 2 SECTIONS.
COMPLETE SECTION 1 ON THE MCQ CARD PROVIDED.
COMPLETE SECTION 2 ON THE ANSWER SCRIPT PROVIDED.
CALCULATORS ARE PERMITTED (ONLY ONE PER STUDENT)
CELL PHONES MAY NOT BE USED AS CALCULATORS
QUESTIONS MAY BE ANSWERED IN ANY ORDER BUT ALL THE SUB-QUESTIONS MUST BE ANSWERED TOGETHER.
DO NOT BE VAGUE IN YOUR DESCRIPTIONS OR EXPLANATIONS ELSE YOU WILL NOT BE AWARDED MARKS.
SUBMIT QUESTION PAPER TOGETHER WITH YOUR ANSWER SCRIPT.

REQUIREMENTS: 1 DOUBLE ANSWER SCRIPT PER STUDENT

/2…

QUESTION 1:

Make 200 ml of a 15 mM Tris, 50 mM EDTA, and 0.03 M NaCl from stock solutions that are 1M Tris, 0.5 M EDTA, and 5000 mM NaCl.

Molecular weights: Tris is 121.1; NaCl is 58.44 and EDTA is 187.0 **[4]**

QUESTION 2

2.1 Cells of genotype *lacI*$^-$ *lacO*$^+$ *lacZ*$^+$ *lacY*$^+$ Hfr are mated with cells of genotype *lacI*$^+$ *lacO*$^+$ *lacZ*$^+$ *lacY*$^+$ F$^-$. In the absence of any inducer in the medium, no β-galactosidase is made. However, when the *lacI*$^+$ *lacO*$^+$ *lacZ*$^+$ *lacY*$^+$ Hfr strain is mated with a strain of genotype *lacI*$^-$ *lacO*$^+$ *lacZ*$^+$ *lacY*$^-$ F$^-$ under the same conditions, B-galactosidase is synthesized for a short time after the Hfr and F$^-$ cells have been mixed. Explain this observation. (BONUS 6)

2.2 Satellites account for approximately 40% of the human genome. Name 4 satellite DNA (full names first if using abbreviations) and provide a brief description of each (12)

[12]

QUESTION 3

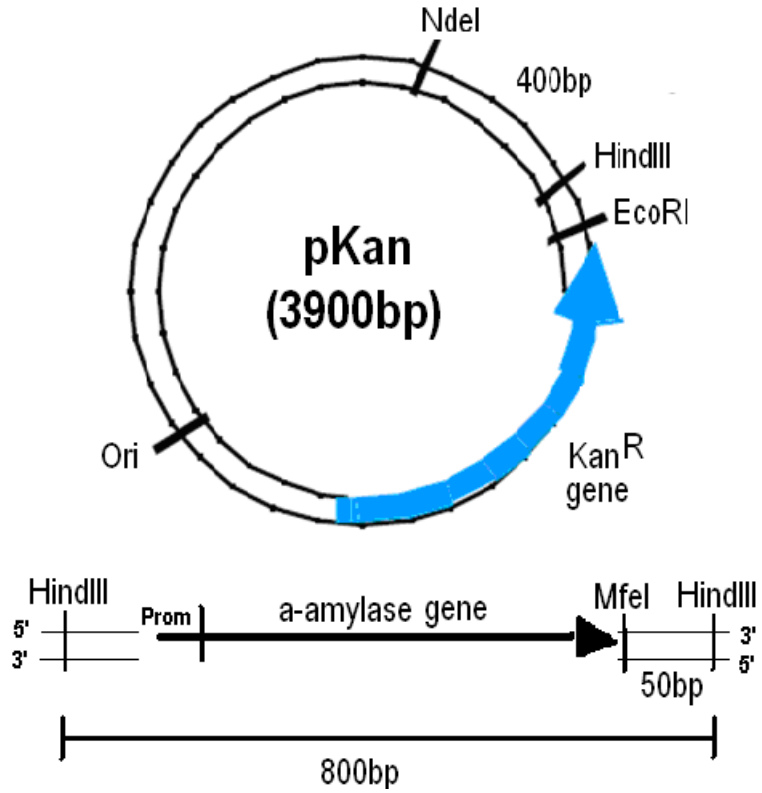
Write 1-12 on your answer script and complete the missing blanks on the CRISPR/Cas prokaryotic defence mechanism.

The CRISPR/Cas prokaryotic defence mechanism involves 3 stages which are _1_, _2_ and _3_. During the initial stage of CRISPR, short sequences knows as protospacers are copied from _4__ and inserted between repetitive sequences such as __5_ within the CRISPR locus within the host genome. _5__ which is the acronym for _6__ _7__ _8__ is responsible for allowing the cas nuclease to differentiate the host sequence from the foreign nucleic acid. The repetitive sequences found in the CRISP array is transcribed into a pre-crRNA by the enzyme _9__ __10__. The transcript is subsequently trimmed by Cas to generate crRNA that contains one spacer and one partial _11__ sequence. The crRNA eventually interacts with the target DNA forming a secondary structure such as the _12__ - loop which will be responsible for recruiting one of the Cas proteins to cleave the target DNA sequence.

[12]

QUESTION 4

You have been provided with a purified DNA preparation of pKan, a 3900bp circular plasmid, which contains a bacterial origin of replication (ori) and the gene for kanamycin resistance (kan^R). In addition, you also have a purified preparation of an 800 bp linear DNA fragment that contains the entire gene for α-amylase. These two DNA molecules with their known restriction enzyme sites are shown below:



Prom: α-amylase promoter

The restriction enzymes listed below cut the following sequences:
*Hind*III: A/AGCTT
*Nde*I: CA/TATG
*Mfe*I: C/AATTG
*Eco*RI: G/AATTC

You want to produce a new recombinant plasmid, which will be called pKanAmy. It will contain genes that confer both kanamycin resistance and amylase expression. To accomplish this task, you have available the four restriction enzymes, *Hind*III, *Nde*I, *Mfe*I, and *Eco*RI.

To begin the cloning process, you digest both pKan and the α-amylase DNA fragment with *Hind*III and separate the DNA samples by size on an agarose gel. You isolate the relevant fragments from the gel, mix them together in a tube and ligate them with the enzyme DNA ligase. You use your ligation mix to transform *E. coli* cells which you then spread on kanamycin-containing plates and grown overnight to isolate bacterial colonies.

Several lab groups in your class independently carried out the following ligation and transformation steps to introduce the *α-amylase* gene into the pKan vector.

Reactions (a) – (f) were carried out in parallel. The results obtained by each of the groups were as follows:

Transformation into Gold cells	# Colonies on LB-Kan Plates			
	Group 1	Group 2	Group 3	Group 4
a. cut pKan + *α-amylase* insert	0	529	2	1125
b. cut pKan (CIAP treated)	0	2	5	930
c. *α-amylase* gene alone	0	0	0	890
d. Uncut pKan	0	852	900	1050
e. 10μl uncut pKan (0.5ng/μl)	0	975	975	1040
f. No DNA	0	0	0	925

Key: CIAP = Calf intestinal alkaline phosphatase
Gold = Stratagene's SoloPack™ Gold supercompetent *E. coli* cells

4.1 Which group obtained the expected/desired pattern of results? (1)

4.2 Provide a likely explanation for the unexpected/undesirable results obtained by each of the other three groups. (Only 1 explanation per group) (3)

4.3 Calculate the transformation efficiency of Stratagene's supercompetent cells. Show formulas & calculations. (3)

4.4 Explain the function of CIAP in cloning? (4)

4.5 Would directional cloning be achieved in your experiment? Explain. (3)

4.6 Now that you have the pKanAmy plasmid containing the α-amylase gene, explain how you would construct a probe that would be used to hybridise and detect the α-amylase gene from a genomic sample by performing a Southern blot. (Note: discuss probe synthesis and detection not Southern blotting) (8)

[22]

QUESTION 5

5.1 In chronic myelogenous leukaemia, name the translocation that gives rise to the Philadelphia Chromosome? (2)

5.2 Briefly explain how the *C-abl* cellular gene of the Philadelphia chromosome becomes oncogenic? (6)

[8]

QUESTION 6

Answer the following short questions.

6.1 Unlike prokaryotes, eukaryotes have three RNA polymerases. What are they and what does each do? (3)

6.2 How does chromatin structure regulate gene transcription? (3)

6.3 Name one trinucleotide repeat disorder. (1)

6.4 Repair of pyrimidine dimers in E.coli by means of RecA utelises which of the repair mechanisms? (2)

6.5 Define fidelity with regard to DNA polymerases. (2)

6.6 Cell lines may be obtained from ATCC. What does this acronym stand for? (2)

6.7 Name one of the next generation sequencing platforms. (1)

6.8 Introducing mutations within a promoter may be used to establish the effect of the polymorphism in gene expression. What is this assay called? (2)

6.9 PCR is used to amplify DNA. Provide the full name a technique used in RNA amplification. (1/2 mark for acronym). (2)

6.10 Which part of the bacterium are disulphide bonds synthesised. (1)

6.11 What do you call the high levels of insoluble protein aggregates formed in E. coli during recombinant protein expression? (2)

6.12 Which sub-stage within meiosis I do homologous chromosomes pair by means of synaptonemal complexes? (1)

6.13 The anti-TRAP operon in *B. subtillis* utilises uncharged tRNA-trp as the metabolite to regulate expression of the anti-TRAP protein. What is the general name given to these mRNA sequences present upstream from the coding sequence that allow for gene regulation. (1)

6.14 Name the complex of proteins binding to the enhancer that interacts with the transcription activators. (1)

6.15 Name an ATP hydrolysis-dependent chromatin remodeling process. (2)

6.16 How is the Dscam gene in *Drosophila melanogaster* able to generate multiple proteins required for multiple connections with adjacent neurons? (2)

6.17 Name the dimeric RNAse enzyme found in eukaryotes that cleaves double stranded RNA molecules into short-interfering RNA. (1)

6.18 What is the name given to the PCR technique that amplifies more than 1 locus simultaneously? (1)

6.19 Provide the full name for X-gal. (2)

[32]