BSc Degree Examinations 2019-20

Department:
BIOLOGY

Title of Exam:
Genome Expression and Maintenance

Time Allowed:
24 Hours (PLEASE NOTE: Late papers will not be marked)

Time Recommended:
2 hours and 30 minutes

Word limit: Please answer the questions within the line limit stated. Content beyond the line limit will not be marked.

Allocation of Marks:
Total marks available for this paper: 100
The marks available for each question are indicated on the paper.

Instructions for Candidates:
All questions should be answered on this question paper using minimum font size Arial 11.
Do not adjust the margin width.
Each question should be answered within the stated line limit.
A note on Academic Integrity

We are treating this online examination as a time-limited open assessment, and you are therefore permitted to refer to written and online materials to aid you in your answers.

However, you must ensure that the work you submit is entirely your own, and for the whole time the assessment is live you must not:

- communicate with departmental staff on the topic of the assessment
- communicate with other students on the topic of this assessment.
- seek assistance with the assignment from the academic and/or disability support services, such as the Writing and Language Skills Centre, Maths Skills Centre and/or Disability Services. (The only exception to this will be for those students who have been recommended an exam support worker in a Student Support Plan. If this applies to you, you are advised to contact Disability Services as soon as possible to discuss the necessary arrangements.)
- seek advice or contribution from any third party, including proofreaders, friends, or family members.

We expect, and trust, that all our students will seek to maintain the integrity of the assessment, and of their award, through ensuring that these instructions are strictly followed. Failure to adhere to these requirements will be considered a breach of the Academic Misconduct regulations, where the offences of plagiarism, breach/cheating, collusion and commissioning are relevant - see AM.1.2.1” (Note this supersedes section 7.3 of the Guide to Assessment).
1.

a) What would happen at the molecular, genomic and cellular level if you deleted 80% of the DnaA boxes at oriC? (5 marks, 6 lines)

b) What would happen at the molecular and genomic level if DnaB was inactivated? (2 marks, 3 lines)

2. An autonomously replicating sequence (ARS) assay with a plasmid containing 5 kb of *S. cerevisiae* DNA produces colonies on selective medium. The 5 kb insert has been sequenced and contains four matches to the ARS consensus sequence (ACS). You have samples of the base plasmid (empty vector), the linear 5 kb *S. cerevisiae* insert on its own, and the original plasmid with the 5 kb genomic insert. How would you determine which ACS(s) is functional? (4 marks, 8 lines)

3. Collisions between the replication and transcription machinery can lead to replication fork collapse before the genome has been fully duplicated. Why do bacteria need strategies to rescue such replication collisions? (2 marks, 3 lines)

4. Cell fusion experiments by Johnson and Roa produced some key findings in our understanding of cell cycle, including the existence of hypothetical factors.

a) Explain why fusion of a cell in G1 phase with a cell in S phase resulted in the G1 nucleus entering S phase. (2 marks, 6 lines)
b) Explain why fusion of a cell in S phase with a cell in G2 phase did not result in the G2 cell entering S phase. (3 marks, 6 lines)

c) Explain why fusion of a cell in G1 phase with a cell in G2 did not result in the G1 nucleus entering S or G2 phase. (2 marks, 4 lines)

d) A group of scientists repeated these cell fusion experiments in the presence of Substance X. In the presence of Substance X, fusion of a cell in G1 phase with a cell in S phase no longer resulted in the G1 nucleus entering S phase. In addition, fusing a cell in interphase with a cell in mitosis no longer resulted in the interphase nucleus entering mitosis. Suggest, with reasons, the possible identity of Substance X. (3 marks, 6 lines)

5. In an experiment to produce a new mutant line, 4 zebrafish embryos (numbered 1-4) are injected with a combination of Cas9 and gRNA targeting the coding region of a gene. An alignment of the sequencing results from the 4 embryos is shown below. The WT sequence is indicated, the gRNA sequence used is underlined, the PAM motif is shown in blue and is boxed:

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>GAACTCAGGAGGTTTCCTGCTGGTTTTCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GAACTCAGGAGGTTTCCT - CTGGTTTTCG</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>GAACTCAGGAGGTTTC - - - CTGGTTTTCG</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>GAACTCAGGAGGTTTCTGGG - TGGTTTTCG</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>GAACTCAGGAG - TTTC - GGG - TGGTTTTCG</td>
<td></td>
</tr>
</tbody>
</table>
a) Which embryos would you choose to raise in order to breed a mutant line and why?  
(3 Marks, 3 lines)

b) Why is there so much variation in sequence between the individuals?  
(1 Mark, 2 lines)

A second "knock in" line is generated by inserting a 400 bp sequence of DNA into another locus. Two PCR primers (indicated as Primer 1 & Primer 2) which produce an amplicon of around 1 Kb in size at the unmodified site are designed along with a gRNA which will cut at the indicated site.

You also make a repair template containing a homologous region around the cleavage site (indicated in blue) as well as the insert DNA sequence (indicated in magenta) as indicated and inject the mixture into zebrafish embryos:

c) You raise three zebrafish lines from the embryos injected with the above cocktail and perform PCR using the primers indicated above. You get the results shown below. Explain the results from each line:  
(3 marks, 4 lines)
6. A group of scientists is working on compound X, which has been found to mimic a ligand of the TGF-β receptor. They analysed the effect of compound X on the cell cycle using flow cytometry.

a) In a normal cell cycle profile produced by flow cytometry, explain why not all cell cycle stages produce their own distinct peak. (4 marks, 8 lines)

b) Scientists performed this analysis on normal mouse cells and cells derived from a mouse tumour. The cells from a mouse tumour did not appear to undergo cell cycle arrest. Explain one possible biological and one possible experimental reason why the treatment had no effect.

Biological Reason: (3 marks, 5 lines)

Experimental Reason: (2 marks, 4 lines)
7. Cells were arrested and synchronized with aphidicolin. A few hours later cells were found to be in G2. These cells were treated with a compound which induced double-strand breaks in the DNA. Underneath is the sequence of the damaged DNA, and the sequence of DNA following repair.

```
5’ --------ATGCTCG  ATGCAAAAGG-------3’          Original Sequence
5’ --------TACGAGC  TACGTTTTCC-------3’
```

```
5’ --------ATGCTGCAAAAGG-------3’                  Repaired Sequence
5’ --------TACGACGTTTTCC-------3’
```

a) Which repair pathway has repaired the damage and how do you know?
   (1 mark, 2 lines)

b) Explain whether this is the expected repair pathway and why.
   (2 marks, 3 lines)

c) The cells used in the experiment were derived from a patient with extensive family history of breast cancer. Explain why these cells may not be a good model for studies on DNA repair.  
   (3 marks, 5 lines)
8. The figures below show levels of ERBB4 protein and mRNA in samples of lung cancer (LC, filled bars) compared to adjacent normal lung tissue (LN, open bars) from 6 patients. ERBB4 is thought to be under the control of miRNA193.

a) Suggest two distinct mechanisms to explain the data that both involve miRNAs.  
   (4 marks, 6 lines)

b) Briefly describe an experiment to test one of your suggested mechanisms given in (a).  
   (3 marks, 5 lines)
9. a) HeLa cells were treated with drug X for 30 minutes before being processed to make nuclear extracts that were subjected to an Electro Mobility Shift Assay (EMSA) using TRE probe (a binding site for AP-1 transcription factor) and antibodies to a number of proteins as indicated. Free probe is not shown. Provide an explanation for the results identifying any controls.  

(4 marks, 5 lines)

b) Summarize the key differences between basal and regulated transcription using specific examples to illustrate your answer.  

(5 marks, 6 lines)

10. Describe how mutations within introns can affect splicing.  

(6 marks, 8 lines)
An experiment was performed to assess whether a stress treatment influences translation efficiency in mammalian cells. Cells were either stress treated or left in control conditions. Polysome profiling was undertaken at 3 and 6 hours after the start of the stress treatment and the ratio of polysomes (P) to monosomes (M) plotted on the graph 1 below. Different letters over the bars indicate statistically significant differences.

Graph 1

a) What conclusions can be made from the data shown in the graph?

(3 marks, 5 lines)
The experiment was repeated using either wild-type cells or cells that expressed a non-phosphorylatable form of eIF2 (S51A). Stress treatment was for 6 hours and the ratio of polysomes to monosomes was plotted on graph 2 below.

Graph 2

b) Provide an explanation for the results shown in graph 2.  

(6 marks, 9 lines)

c) Suggest an experiment to confirm your explanation for part (b). Describe the expected outcome.

(3 marks, 6 lines)
Consider the scenarios in the table below and state in the right column whether the expression of the target gene in the expression plasmid occurs or not.

(6 marks)

<table>
<thead>
<tr>
<th>IPTG</th>
<th>lac operator upstream of T7 RNA polymerase gene</th>
<th>lac promoter upstream of T7 RNA polymerase gene</th>
<th>T7 promoter</th>
<th>Target gene expression?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>Mutation affecting LacI binding</td>
<td>Wild type</td>
<td>Wild type</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Mutation affecting T7 RNA pol binding</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>Wild type</td>
<td>Mutation affecting RNA pol binding</td>
<td>Wild type</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>Wild type</td>
<td>Mutation affecting RNA pol binding</td>
<td>Wild type</td>
<td></td>
</tr>
</tbody>
</table>
13. 

a) How do mutations in the inducer-binding site of the LacI repressor affect the expression of the lac operon? (3 marks, 5 lines)

b) Explain why mutations that eliminate LacI function are recessive. (2 marks, 5 lines)

c) What do mutations in the palindromic motif of the operator lead to? (2 marks, 3 lines)

14. You perform an Ames test with two mutagenic compounds (NaN₃ and EthBr) and two strains of His Salmonella typhimurim plated on histidine deficient media as indicated in the figure below. You incubate the mutagenic compounds with and without liver cell extract as indicated in the table, spot the mixture onto filter paper in the middle of the plates and get the results indicated in the figure (black dots represent individual colonies).

<table>
<thead>
<tr>
<th></th>
<th>NaN₃</th>
<th>+</th>
<th>+</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>EthBr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Liver Extract</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

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a) What do the results tell you about the mutations which cause the lack of histidine in the different strains? Justify your answer.  

(2 Marks, 5 lines)

b) Explain how incubation with liver extract affects the mutagenic potential of the different compounds.  

(2 Marks 5 lines)

15. MyoD is a basic helix loop helix (bHLH) transcription factor which is capable of dimerising with other HLH transcription factors, such as E47. It binds to DNA motifs called E-boxes to activate the transcription of downstream genes.

In an experiment to determine whether heterodimerisation with E47 increases the synergistic activation of promoters by MyoD, constructs expressing MyoD-MyoD dimers or MyoD-E47 heterodimers were produced as in the diagram below and transfected into cultured cells. Constructs expressing MyoD or E47 alone were also produced.

The cultured cells contained a reporter containing E-boxes (4R-MCK), as well as a constitutively active construct, pCG1. Gene expression was measured by RT-PCR. 4R-MCK should only be expressed when activated by a factor capable of binding E-boxes, whereas pCG1 should be constitutively expressed.
The following results were obtained:

- - - - - - +  His-GAL4-VP16
- - - - - + -  FL-MyoD / E47
- - - - + - -  FL-MyoD / MyoD
- - + + - - -  FL-E47
- + - + - - -  FL-MyoD

Explain these results (4 marks, 8 lines)