VICTORIA JUNIOR COLLEGE
BIOLOGY DEPARTMENT
JC2 PRELIMINARY EXAMINATIONS 2017
Higher 3

CANDIDATE NAME

CLASS | | | | | | INDEX NUMBER | | |

BIOLOGY 9815/01

25 September 2017

Additional Materials: Answer Paper **2 hours 30 minutes**

READ THESE INSTRUCTIONS FIRST

Write your CLASS/ INDEX no. and name on all the work you hand in.
Write in dark blue or blue pen.
You may use a soft pencil for any diagrams, graphs or rough working.
Do not use any staples, paper clips, highlighters, glue or correction fluid.

Section A
Answer **all** questions.

Section B
Answer **three** out of **four** questions.

Section C
Answer the question.

At the end of the examinations,
1. Fasten all your work securely;
2. Circle the number of the section B question you have answered in the grid opposite.

For Examiner's Use	
Section A	
1	
2	
3	
4	
5	
Section B	
6	
7	
8	
9	
Section C	
10	
Total	

The number of marks is given in brackets [ ] at the end of each question or part question.

Section A

Answer all questions in this section.

1 **(a)** A genetic study of coat type in purebred dogs has identified a mutation in the keratin gene KRT71, associated with the curly fur phenotype in some breeds.

Keratin is known to be abundant in cysteine. Suggest how a single mutation in KRT71 can lead to this curly fur phenotype. [2]

(b) Mandy decided that she wanted to loosen the curls in her poodle's fur. She first hosed her poodle down with water and then applied an ammonium thioglycolate solution (a reducing agent) as she rolled her poodle's fur around larger-diameter rollers. Next, she applied hydrogen peroxide (an oxidising agent) on the fur to set the curls. She was pleased with the results.

Explain how this works at the molecular level. [2]

(c) Mandy was warned by the vet never to feed her poodle chocolates. Dogs cannot metabolize theobromine found in chocolates as well as humans can. For example, it will take a dog 17 ½ hours to metabolize and excrete just half of the theobromine it has ingested. It can thus accumulate in the animal's body to a level that can be toxic or even fatal.

Theobromine is a natural compound found in the cocoa bean and other plants. Genes of the cytochrome P450 family code for enzymes that are involved in detoxification of drugs and compounds such as theobromine. Human enzymes work twice as fast as canine enzymes.

Suggest the advantage of possessing this enzyme and discuss where this discovery places humans and non-canine mammals on the phylogenetic tree with dogs. [4]

(d) Mandy discovered that her poodle is suffering from biotin deficiency. This is a rare nutritional disorder which can become serious, even fatal, if allowed to progress untreated. Biotin is part of the B vitamin family. Mandy realised that her poodle's biotin deficiency can be caused by consuming raw egg whites over a period of time. Egg whites contain high levels of avidin, a protein that binds biotin strongly.

In fact, this strong avidin-biotin interaction has been exploited in antigen detection in medical research. State the advantage and outline how such an immunoassay works. [2]

[Total: 10]

2 Human tau is encoded by the MAPT gene, located on chromosome 17. Tau is the major microtubule associated protein (MAP) of a normal mature neuron. It is found naturally as six molecular isoforms in the human brain. An established function of MAP is their interaction with tubulin and promotion of its assembly into microtubules and stabilization of the microtubule network.

(a) **(i)** Suggest how these different six isoforms of the Tau protein can be formed from a single gene. [1]

(ii) Explain the biological significance of the mechanism in (i) in normal physiology. [1]

Alzheimer's disease and related neurodegenerative diseases are collectively known as tauopathies as they are characterized by the presence of aggregates of Tau. Tau protein is abnormally hyperphosphorylated and aggregated into bundles of filaments.

(b) Explain how hyperphosphorylation may play a role in the aggregation of Tau protein. [2]

Tau protein consists of unusually high levels of hydrophilic amino acids. Because of its hydrophilic character, it does not adopt the compact folded structure typical of most cytosolic proteins. Instead the entire Tau molecule is shown to be 'natively unfolded' or intrinsically disordered. This means that the polypeptide is highly flexible and mobile.

(c) **(i)** Explain the principles that regulate the compact folding of typical cytosolic proteins. [3]

(ii) Suggest the biological significance of these characteristics of Tau protein. [1]

(d) Scientists conducting research on Tau protein were able to extract much information of the protein function from its gene sequence. Explain the benefits of such applications with reference to biological and medical communities. [2]

[Total: 10]

3 Six percent of the proteins in the yeast genome show no homology in their sequences when compared with proteins of known function in other organisms (Fig. 3).

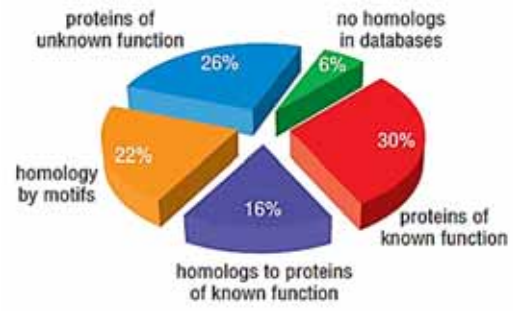


Fig. 3

(a) Protein **Y** is one such protein. Rationalise how two named methods can be used to predict its function. [2]

(b) Outline a named one-step method that can be used to purify yeast proteins that have been expressed in *E. coli*. [4]

(c) Chronic myelogenous leukemia (CML) and variants of acute lymphoblastic leukemia (ALL) are blood cancers that arise from a chromosomal translocation resulting in a constitutively active Bcr-Abl tyrosine kinase.

Suggest how you will design a RNAi-based strategy to treat this type of cancers. [2]

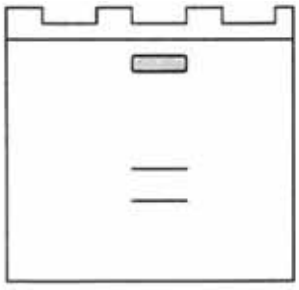
(d) Describe one advantage and one limitation each, of fluorescent and chromogenic detection labels, used in immunohistochemistry. [4]

[Total:12]

4 A protein from bacteria *Thermococcus* species that lives in a thermal vent has been isolated. You obtained the gene sequence and synthesized the protein in *Escherichia coli* bacteria that grow at room temperature.

(a) Explain briefly why and how the gene sequence of the desired protein and *E.coli* were used for the study of the protein structure [3]

During the purification process, you heated the *E.coli* cells to 90^{o}C and then subjected the soluble portion of the heated mixture to native polyacrylamide gel electrophoresis (PAGE). After applying Coomassie stain to the gel, you obtained the gel as shown below.



(b) Suggest why the purification process involved the heating of the *E.coli* cells to 90^{o}C. [1]

(c) Give reasons why your procedure will not allow you to accurately determine the molecular weight of your protein. [3]

(d) Nevertheless, you want to isolate the protein so that you can further elucidate its structure and function. State one method which allows you to do so without affecting its structure or function. [1]

(e) Your lab assistant helps you isolate the protein based on your method in **(d).** Upon isolation of the protein, he incubates half of your protein sample at room temperature (25^{o}C) overnight and then applies it to an SDS-PAGE gel. He finds the protein no longer migrates as one band, but many. However, the other half of the protein sample, which he had left overnight at 80^{o}C, still migrates as one single band in the non-SDS gel. Explain this observation. [2]

[Total:10]

5 Proteins rarely act alone as they are built from a large number of protein components organized by their protein–protein interactions.

(a) Define protein-protein interactions. [1]

(b) Describe the protein-protein interactions between

(i) the subunits of a named protein. [2]

(ii) named proteins in a complex. [2]

(c) Aberrant protein-protein interactions are the basis of multiple aggregation-related diseases such as Alzheimer's disease. Suggest how multiple aggregation of proteins in aberrant protein-protein binding can result in diseases such as Alzheimer's. [3]

[Total: 8]

Section B

Answer 3 out of the 4 questions in this section.

6 **(a)** p53 is a well-known tumour suppressor protein with a proline-rich region implicated in initiating cell apoptosis via interaction with different promoters. Suggest the structural and functional significance of having an abundance of proline in these regions. [4]

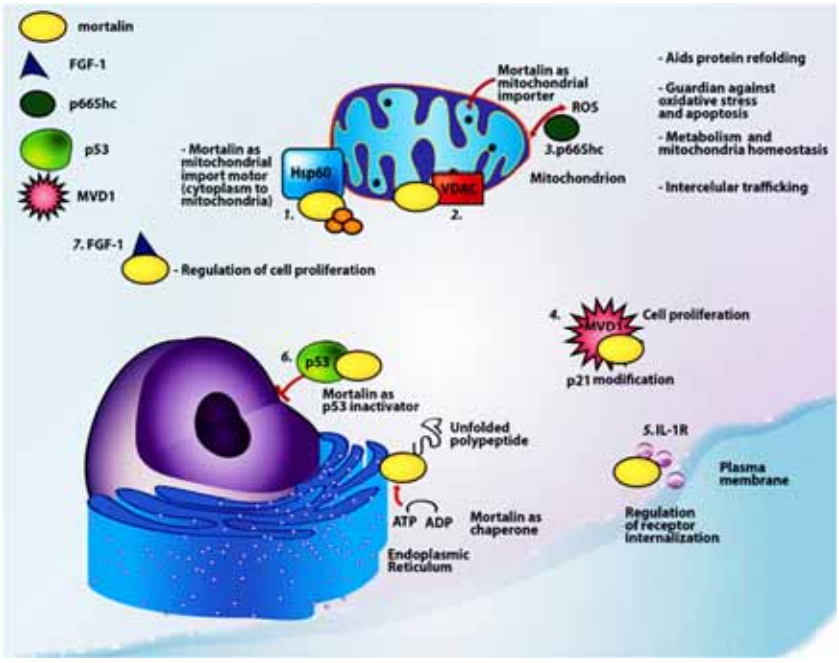
The N-terminal proline-rich domain of human p53 has been shown to be important for the induction of apoptosis. However, the corresponding region in mouse and other species is not highly conserved. Research efforts to confirm this domain's role in eliciting cell death have been inconclusive. While one mutant missing a large proportion of this proline-rich domain is selectively defective in causing cell death in the mouse, other mutants display wildtype phenotype in all assays performed.

(b) What conclusions can currently be drawn here about the importance of this region for the purpose of tumour suppression? [2]

(c) The p53 DNA-binding domain recognizes specific regulatory sites on the DNA. Suggest how this is achieved. [2]

(d) Mortalin, a member of the heat shock protein (HSP) 70 family, is overexpressed in different tumour types. It is thought to contribute to the process of carcinogenesis by multiple ways.

With reference to the figure below and your knowledge of how cancer arises, suggest two ways in which mortalin may work to promote the development of cancerous cells. [2]



(Source: http://www.mdpi.com/biomolecules/biomolecules-02-00143/article_deploy/html/images/biomolecules-02-00143-g002.png)

[Total: 10]

7 In order to investigate the subcellular locations of proteins **1**, **2** and **3**, rat liver cells were homogenised and the cellular components separated into cytosol and lysosomes by centrifugation. The temperature during these steps was maintained between 0-4°C.

The components were then subjected to 2D gel electrophoresis and the results are shown in Fig. 7.1 and Fig. 7.2.

Fig. 7.1

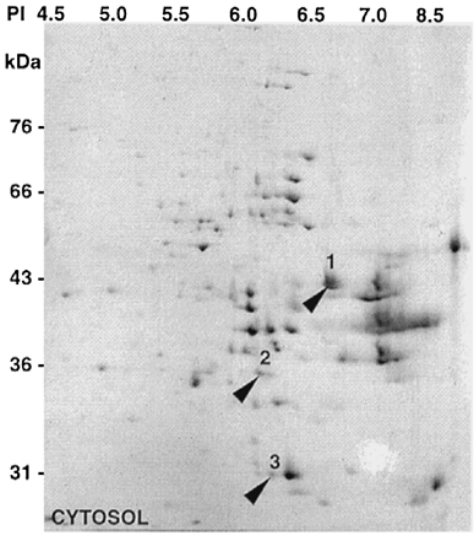
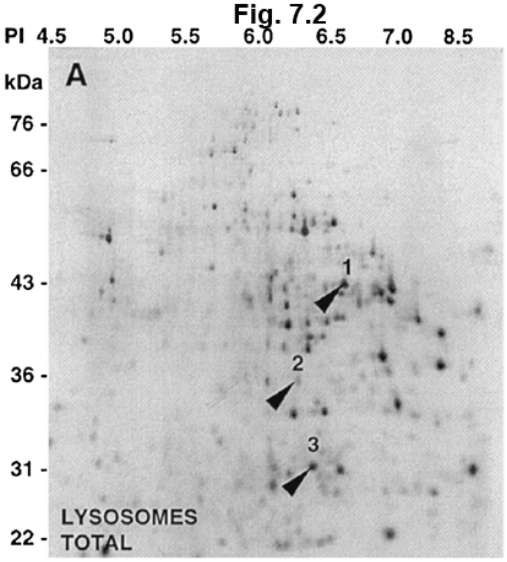


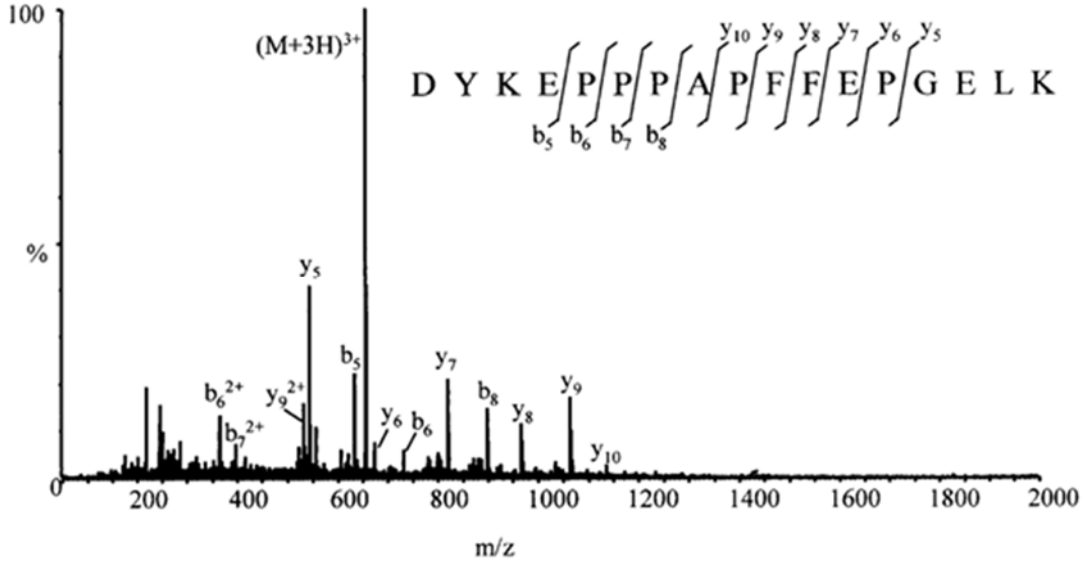
Fig. 7.2



(a) Explain the principles behind 2D gel electrophoresis. [3]

(b) The pH of lysosomes is usually 4.5, and yet Fig. 7.2 shows that proteins are not clustered around pH 4.5 but are instead spread across the entire pH gradient of the gel. Comment. [2]

The spots which are labelled 1, 2 and 3 were identified as proteins 1, 2 and 2 when the spots were excised, subjected to trypsin digestion and then fed into tandem mass spectrometry (MS/MS). The mass spectrum results of one of the peptides are shown in Fig. 7.3.



Analysis of a peptide fragment by MS/MS. The C-terminal (y-type) or N-terminal (b-type) fragments produced are due to the way that the peptide is fragmented.

Fig. 7.3

(c) Describe briefly the principles of mass spectrometry, and state exactly what is measured. [3]

(d) Explain why Fig. 7.3 shows two different peaks for fragment y_9 [1]

(e) Name another method which can be used to determine amino acid sequence, and state one advantage of this method over using MS/MS. [1]

[Total: 10]

8 **(a)** Multi-protein complexes are critical to biological processes inside cells. Proteins that have not been functionally annotated can be determined based on their role as components of complexes. Multiprotein complexes can be purified by affinity purification and then analysed by mass spectrometry.

Describe how tandem affinity can be used to examine large scale *in vivo* protein-protein interactions of a protein named **FUNK**. [4]

(b) Identify one advantage and one disadvantage of using epitope tagging in the isolation of protein complexes. [2]

(c) How does DNA transposon strategy differ from RNAi in characterising gene function? [2]

(d) To generate the stable expression of siRNAs in mammalian systems, mammalian expression vectors must be able to direct the intracellular synthesis of target-specific siRNAs.

State how stable and appropriate expression is made possible. [1]

(e) State the strengths underpinning immunohistochemistry in studying protein function. [1]

[Total: 10]

9 **(a)** Platelet-derived growth factor is received by a fibroblast cell via its receptor tyrosine kinase (RTK). The ligand-bound RTK activates a SH2 domain-containing protein, which in turn activates the G protein Ras. Activated Ras then activates the first out of three serine-threonine kinases of the mitogen-activated protein (MAP) kinase pathway. All three kinases in this pathway activate multiple substrates at each step. The final enzyme in the pathway causes a change in gene transcription, which results in the proliferation of the fibroblast for blood vessel formation.

Account for the conformational changes that occur to RTK during signal reception. [2]

(b) Explain what a protein domain is and the role of the SH2 domain in its interaction with the activated RTK. [2]

(c) Outline the conformational changes and interactions involving the G protein Ras. [2]

(d) Discuss the significance of the MAP kinase pathway in cell signalling. [2]

(e) Numerous lines of evidence support a role for RTKs in the growth and progression of human malignancies.

Suggest how selective SH2 inhibitors can be used to treat cancer. [2]

[Total: 10]

Section C

Answer the question in this section.

10 **(a)** Fig. 10 shows the workflow involved in the identification of Protein **S** (boxed band) from a protein mixture extracted from a tissue.

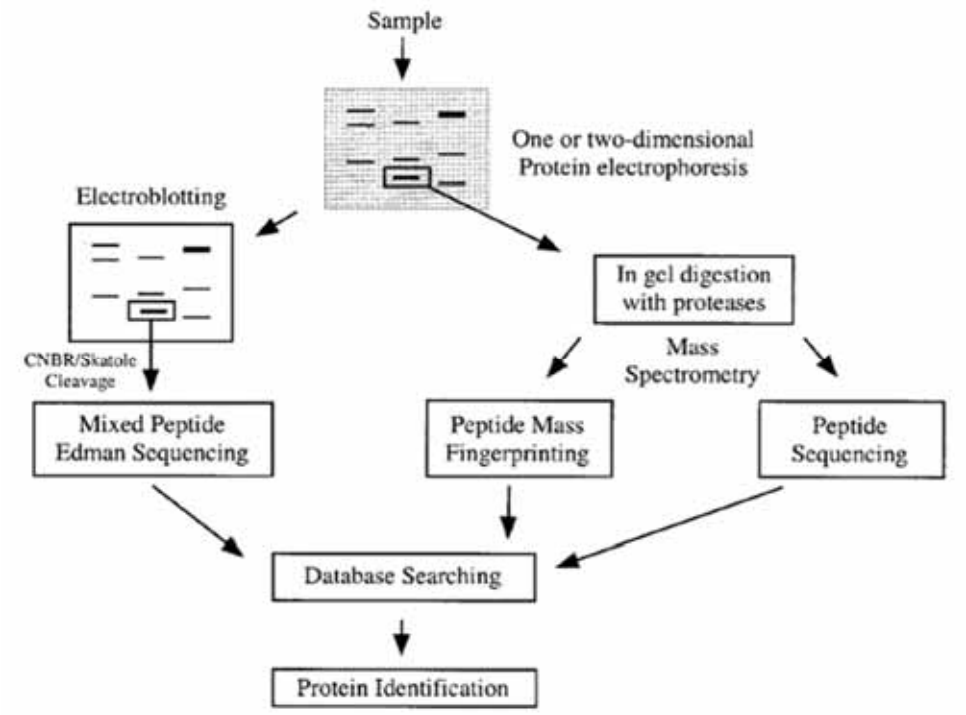


Fig. 10
(Source: Molecular Biologist's Guide to Proteomics.)

After the full sequence of protein **S** has been determined following database search of its peptide mass fingerprint, what can be done to predict its function using an *in silico* approach? [8]

(b) You are the leader of an R&D laboratory. Your task is to investigate how a potential drug, Molecule **D**, interacts with a protein kinase that has been implicated in the signalling pathway in cancer.

Outline a strategy to generate the 3-dimensional structure of this protein-drug interaction that will aid drug design. [6]

(c) With reference to three named examples, explain how protein modification or cleavage is involved in gene expression and its regulation. [6]

[Total: 20]