UNIVERSITY OF CAMBRIDGE

NATURAL SCIENCES TRIPOS PART IA BOC/WP

Biology of Cells – Practical

Friday 10th June 2022

*Answer **all questions.***

All questions carry equal marks.

You have 3 hours (plus any pre-agreed individual adjustment) to answer this paper.

Please treat this as an open book exam and write your answers within the time-period. Uploading time should not be included in the allocated exam time.

Where appropriate (e.g. for formulae, equations or diagrams) you may hand write and submit scans or photographs of your answers. Candidates should not copy and paste figures from any other documents.

For all questions, please combine any text and figure files into a single file, for example pdf.

For reference, a list of the questions is given below.

The average suggested time for each question is 15-20 minutes.

1. Microscopy
2. Protein structure
3. Quantification of DNA and protein
4. Enzyme activity
5. Photosynthesis and the oxygen electrode
6. Fungal genetics
7. Drosophila genetics
8. Polymerase chain reaction (PCR)
9. The embryological zoo

1. Microscopy

While going through your microscope samples you notice that the label identifying one of them is partially damaged and you can only read ‘stained with haematoxylin and eosin’. Curious to identify the specimen and correct the label you decide to investigate.

a) What type of microscopy technique could you use, and what adjustments would you make to microscope controls A and B (see Figure 1), to obtain the optimum image of the specimen using a 40x lens?

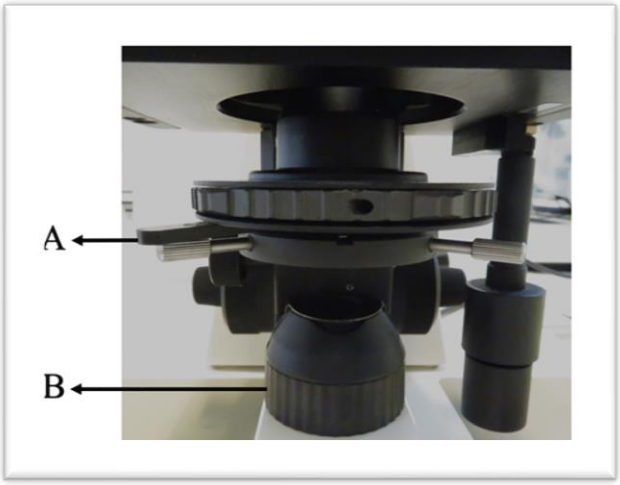

Figure 1. Microscope used to observe the unknown specimen.

b) With the microscope correctly adjusted, you realise a small section of your specimen is outside the field of view. Which microscope control(s) could you use to bring the whole specimen into view?

c) After performing the adjustments, you are able to obtain the image of the specimen shown in Figure 2. Identify the organism and the feature labelled as A within the dotted circle.

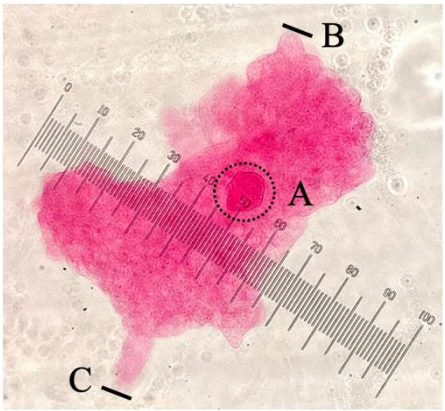

Figure 2. Micrograph of the unknown specimen viewed with the 40x lens. The scale is provided by the microscope graticule.

[Question continues on the next page]

d) Briefly explain how you would estimate the length between B and C in Figure 2.

e) Recent work has shown that in organisms of the same phylum as your specimen, and also in mammalian cells, the vacuole membrane protein 1 (Vmp1) is required for the integrity of rough endoplasmic reticulum (RER). Figure 3 shows images of RER in a wild type cell and a cell with a mutation in the *vmp1* gene. What type of microscopy technique and sample preparation were used to collect the images?

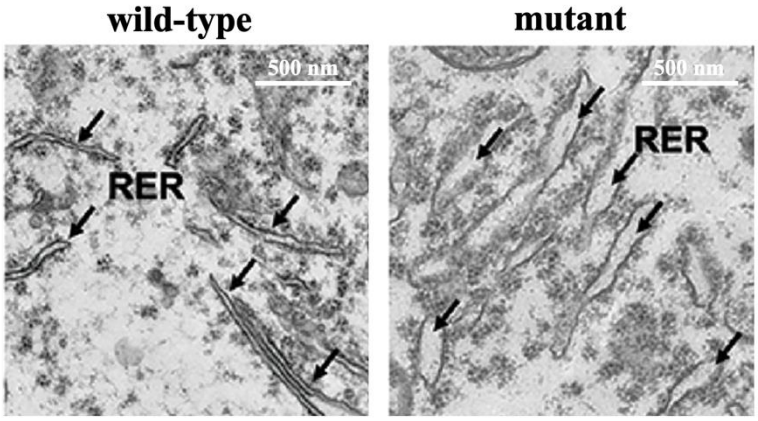

Figure 3. Representative micrographs of RER in wild-type (left) and *vmp1* mutant (right) cells. Scale bar represents 500 nm. The arrows indicate individual RER tubules.

f) You would like to understand to what extent the *vmp1* mutation affects RER structure. Briefly explain how you would calculate the average width of the RER tubules in each cell type in Figure 3. What is the ratio between the width of the RER tubules in wild type compared with the *vmp1* mutant?

g) Knowing the *vmp1* mutation alters RER structure, you wonder whether it may also affect the distribution of RNA in the cells. Describe how you would use light microscopy to determine whether the cells in the mutant show changes in the distribution of RNA? Give details of how you would prepare the cells and what control(s) you would perform.

2. Protein structure

Gram negative bacteria have an outer and an inner membrane with a periplasmic region between them. The outer membrane has a protective function for these bacteria, but as the cells need to exchange solutes with the environment, proteins form pores to enable the uptake of nutrients across the outer membrane. One such protein is Outer Membrane Protein A (OmpA), whose structure is shown in Figure 4.

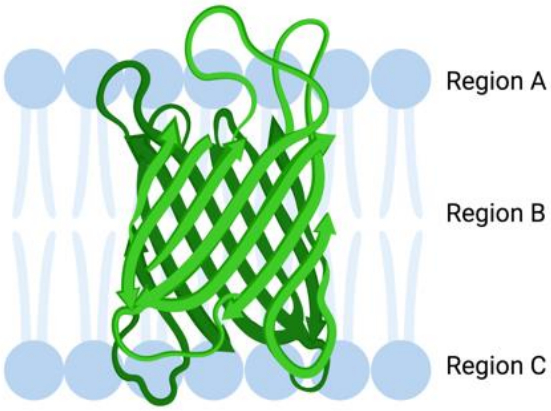

Figure 4. Structure of OmpA shown residing in the outer membrane. The blue shapes represent the membrane phospholipid bilayer.

a) What is the predominant secondary structure seen in OmpA? Sketch a Ramachandran plot for OmpA to validate your answer.

b) Name three amino acids that are more likely to be found in the part of OmpA residing in contact with region B of the outer membrane.

c) Name three amino acids that are more likely to be found in the part of OmpA residing in regions A or C of the outer membrane.

d) Describe a protein secondary structure not seen in OmpA**.**

3. Quantification of DNA and protein

You are working as a summer student in the Cereal Symbiosis laboratory where you are investigating rice. You have carefully extracted DNA and protein separately from four experimental samples, giving eight tubes in total.

a) Describe the approach that you would take in order to extract soluble protein from rice leaf tissue. Ensure that you list key pieces of equipment and solutions that are required to do this.

You have accidentally wiped off the labels from your tubes. To work out which samples are which, you decide to quantify the amount of DNA and protein in each tube using spectrophotometry.

First you remove 10 µl from each tube and add this to 990 µl of water. You add this to a cuvette of 1 cm path length and use a spectrophotometer to read the absorbance at 260 nm (A_{260}). These data are shown in Table 1. Assume that ε for DNA at 260 nm is 20 litres cm-1 g-1.

Table 1. Spectrophotometry A_{260} data from the samples.

Tube	1	2	3	4	5	6	7	8
A_{260}	0	0.097	0.055	0.002	0.001	0.087	0.076	0

b) Calculate the concentration of DNA in the original eight samples in mg/ml. Which tubes do you think contain the DNA samples?

Next you prepare a dilution series of bovine serum albumin (BSA) and remove 50 µl from each dilution, which you add to 950 µl of Bradford reagent. Using a cuvette you measure the absorbance at 595 nm (A_{595}) and record the data, which are shown in Table 2.

Table 2. Spectrophotometry A_{595} data for BSA standard curve samples. These data are plotted below in Figure 5.

BSA concentration (µg/ml)	0	100	200	300	400	500	600	700
A_{595}	0.00	0.15	0.30	0.42	0.51	0.59	0.63	0.65

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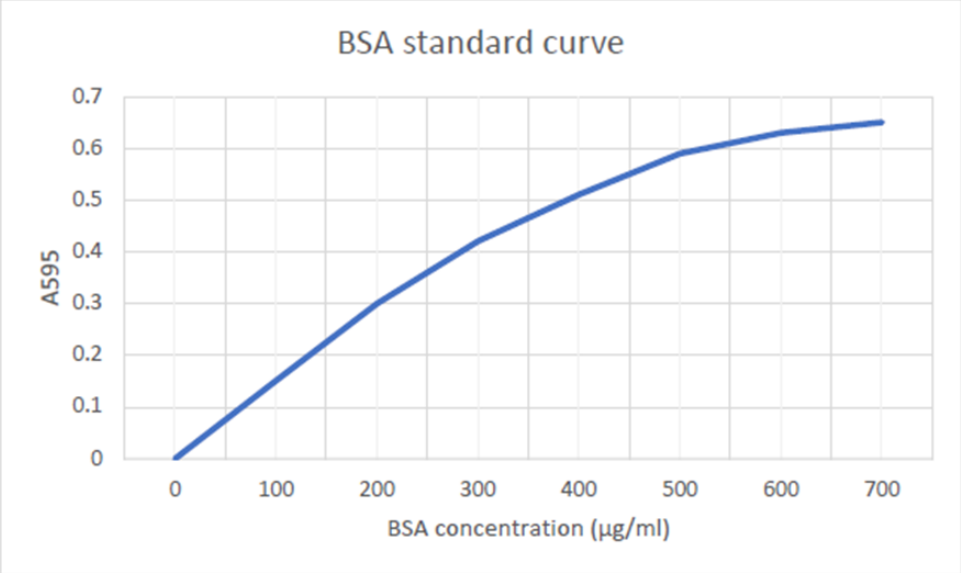

Figure 5. BSA standard curve A_{595} data. Data from Table 3 are plotted.

Next you repeat measurement of your samples by adding 50 µl to 950 µl of Bradford reagent. These data are shown in Table 3.

Table 3. Spectrophotometry A_{595} data for your experimental samples.

Tube	1	2	3	4	5	6	7	8
A_{595}	0.52	0.02	0.01	0.40	0.61	0.01	0.00	0.60

c) Using the provided standard curve in Figure 5, Calculate the amount of protein (in µg) in 50 µl of each of the eight samples added to the Bradford reagent. Which tubes do you think contained the protein samples?

d) How does Bradford reagent work in this assay for measuring protein concentration? What are the limitations of the reagent/assay and how does this relate to your measurements?

e) How could you change the experimental settings to resolve these limitations?

4. Enzyme activity

Deoxyribonucleoside kinases (dNKs) catalyse phosphorylation of 2'-deoxyribonucleosides to 2'-deoxyribonucleoside monophosphates (dNMP) using ATP as the phosphate donor, as shown in Figure 6.

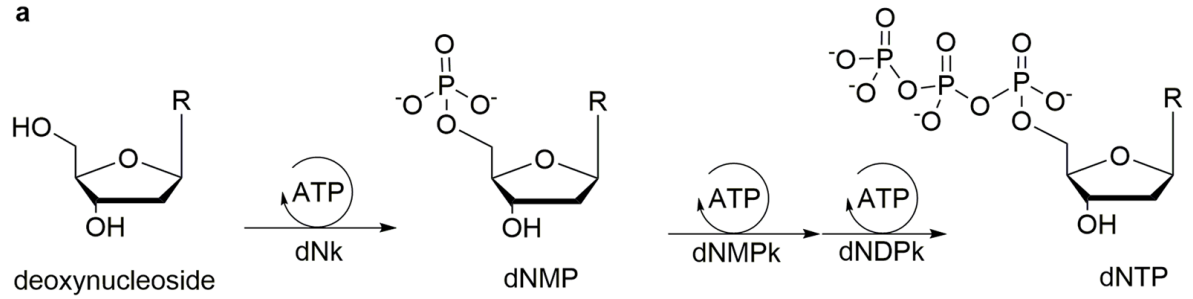

Figure 6. Phosphorylation of 2'-deoxyribonucleoside to 2'-deoxyribonucleoside monophosphates (dNMP) by dNK.

This is the rate-limiting step in the production of deoxyribonucleotide triphosphates (dNTPs) from deoxyribonucleosides, which are used for the synthesis of DNA. You discover a new 29 kDa dNK and you want to assess its kinetic properties and substrate specificities.

a) How many moles of dNK enzyme will you have in 1 µg of pure protein?

To determine the substrate specificity of this enzyme you perform a reaction with 100 ng of dNK and four 2'-deoxyribonucleosides. You obtain the K_m and V_{max} values listed in the Table 4 below.

Table 4. dNK kinetic analysis.

	K_m (µM)	V_{max} (pmoles product/min)
deoxythymidine	50	88
deoxycytidine	60	102
deoxyguanosine	2000	80
deoxyadenosine	373	215

b) From Table 4, which 2'-deoxyribonucleoside is the highest affinity substrate of dNK?

k_{cat} refers to the turnover rate of the enzyme (the number of substrate molecules catalyzed per enzyme molecule per unit time). It is expressed in units of s^{-1} and is calculated as:

$$k_{cat} = V_{max}/\text{total moles of enzyme}$$

c) What is the k_{cat} for dNK and deoxycytidine, assuming that 100% of your enzyme is active?

[Question continues on the next page]

To further characterise your new dNK you apply two inhibitors to your enzyme, a competitive inhibitor and a non-competitive inhibitor, but you can't remember which inhibitor was in which tube. You obtain new K_m and V_{max} values for 100 ng of dNK and deoxycytidine as follows in Table 5.

Table 5. Analysis of inhibitor action on dNK.

	K_m (µM)	V_{max} (pmoles product/min)
Inhibitor A	107	104
Inhibitor B	24	32

d) Which inhibitor is the competitive inhibitor, and which is the non-competitive inhibitor?

You are given another 2'-deoxyribonucleoside, deoxyinosine, to test whether it is an inhibitor of your dNK. For this test you add increasing amounts of deoxyinosine to a reaction containing dNK and deoxycytidine. You measure the phosphorylation of deoxycytidine by the formation of product (dCMP) and you obtain the curve shown in Figure 7.

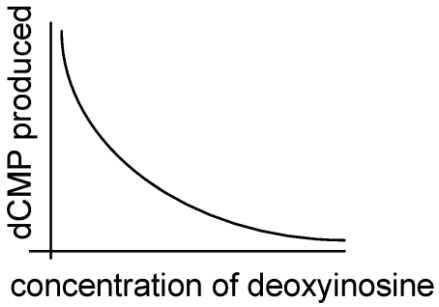

Figure 7. Production of dCMP by dNK in the presence of deoxyinosine.

e) Can you tell from this experiment if deoxyinosine is an inhibitor of dNK and if so, which type of inhibitor it is?

While your dNK phosphorylates 2'-deoxyribonucleoside, it does not phosphorylate ribonucleosides such as cytidine. To understand this specificity, you perform X-ray crystallography with your dNK bound to 2'-deoxycytidine (dCyd). Figure 8 shows some structural details of the active site. The 3'-OH of dCyd is seen to form two hydrogen bonds (dotted lines) with Glu172 and Tyr70 of dNK.

[Question continues on the next page]

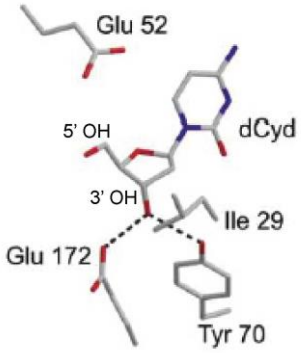

Figure 8. The active site of dNK bound to 2'-deoxycytidine.

f) From the structural information in Figure 8, why do you think cytidine is not a substrate for this enzyme?

g) Your dNK appears to be inhibited by dTTP. Why might this be a useful property of your enzyme for the cell?

5. Photosynthesis and the oxygen electrode

From a forward genetic mutagenesis screen you have isolated a new Arabidopsis mutant that shows a pale green phenotype. You have named the mutant *defective in photosynthesis1* (*dip1*). To investigate the role of *DIP1* you decide to measure photosystem activity using an oxygen electrode.

You use 8 grams of seedlings for either wild type or *dip1* mutants and homogenize them in 30 ml of 0.4 M sucrose buffer. The homogenate is then centrifuged at 3000 *g* and the chloroplast pellet is resuspended in 4.5 ml of sucrose buffer.

400 µl of the chloroplast suspensions were removed and added to 900 µl of acetone. 1000 µl of this was used to measure absorbance at 652 nm (A_{652}) using a spectrophotometer. Multiplying the A_{652} value by 2.9 can be used to calculate the concentration of chlorophyll in mg^{chl} ml^{-1}. The A_{652} measurement from wild type was 0.51 and *dip1* was 0.28.

a) Calculate chlorophyll amounts (mg^{chl}) in the acetone extractions from wild type and *dip1* seedling extracts.

200 µl of the resuspended chloroplasts were placed in an oxygen electrode containing KCN and the electron acceptor ferricyanide. The final volume in the electrode chamber was 1 ml. The change in oxygen tension in the electrode over a time-course was measured.

These data are shown in Table 6.

Table 6. Oxygen electrode measurements from wild type and *dip1* chloroplast extractions. Lights were turned on immediately after the dark measurement at 120 seconds. An electrode reading of 1 mV is equivalent to 2.55 x 10^{-4} O_2 µmoles ml^{-1}.

Light/Dark	Time	Wild type (mV)	*dip1* (mV)
Dark	0	399	390
Dark	30	405	406
Dark	60	425	408
Dark	90	430	415
Dark	120	432	422
Light	150	653	438
Light	180	768	511
Light	210	873	533
Light	240	944	552

b) Calculate photosynthetic rate in wild type in µmole O_2 min^{-1} mg^{chl-1}.

c) Calculate photosynthetic rate in *dip1* in µmole O_2 min^{-1} mg^{chl-1}.

[Question continues on the next page]

d) What can you conclude from these data about photosynthesis in *dip1* as compared to wild type with respect to photosystem I versus II?

e) From your genetic screen you have isolated a second mutant called *dip2*, which shows a similar pale green phenotype. Both *dip1* and *dip2* are recessive. Describe a genetic experiment where you could establish whether *dip1* and *dip2* are mutant alleles of the same genes, or represent mutations in different genes?

6. Fungal genetics

You have isolated a *Saccharomyces cerevisiae* strain that is unable to grow on medium lacking adenine, and accumulates red pigment if adenine is limiting. The mutation responsible for this phenotype is a point mutation in the *ADE2* gene at position 135 of the ORF that introduces a STOP codon. You name the allele *ade2-135*.

In order to study the genes involved in the biosynthetic pathway of adenine upstream of *ADE2*, you decide to mutagenize the strain containing *ade2-135* and screen for colonies that are white on a medium containing a limiting amount of adenine.

a) Explain the rationale of this approach.

In your screen, you obtain a single clone with the desired phenotype (i.e. white on limiting adenine). Before analysing this clone in detail, you need to verify that this phenotype is not due to the reversion of the mutation in the *ADE2* ORF.

b) Propose the simplest way to test that this is not the case.

Having verified that the mutation is in a different ORF than *ADE2*, you need to understand if the phenotype is due to a mutation of one or multiple genes. You decide to cross the newly generated multi-mutant strain with a strain of the opposite mating type containing the *ade2-135* mutation, but that is otherwise wild type.

c) What will be the colour of a colony of the diploid on a medium with a limiting amount of adenine if the clone isolated from the screen has a single mutation outside the *ADE2* ORF?

d) What will be the colour of a colony of the diploid on a medium with a limiting amount of adenine if the clone isolated from the screen contains 2 new mutations (outside the *ADE2* ORF)?

You then analyse the progeny of this cross by allowing the diploid to sporulate.

e) If the new mutation inactivates a single gene, assuming the gene is NOT linked to *ADE2*, what proportion of spores will give rise to white colonies in a medium with limited amount of adenine?

f) What proportion of spores will be *ade*- i.e. unable to grow on medium lacking adenine?

g) If you have 2 new mutations that inactivate 2 genes, both upstream of *ADE2*, and assuming these genes are NOT linked to each other, what proportion of spores will give rise to white colonies in a medium with limited amount of adenine? Justify your answer.

[Question continues on the next page]

h) If you have 2 mutations that inactivate 2 genes, one upstream and the other downstream of *ADE2*, assuming the 3 genes are NOT linked to each other, what proportion of spores will give rise to white colonies in a medium with limited amount of adenine? Justify your answer.

From your analysis, you conclude that the white clone from your screen contains a single mutation outside *ADE2* ORF in a gene that you name *ADE-X*. Your next task is to understand if *ADE2* and *ADE-X* are linked. For this purpose, you cross your mutant with a wild type strain of *S. cerevisiae*, you grow the diploid, put it in starvation conditions to induce meiosis and sporulation and analyse 5,000 spores.

This is the result you obtain:

Red colonies: 1,298

White colonies: 3,702 (of those, 1,244 are able to grow on a plate without adenine)

i) From those results, what can you conclude about the linkage between the 2 genes? Justify your answer.

j) If the 2 genes were at a genetic distance of 10 cM, how many red colonies would you expect?

7. Drosophila genetics

After mutagenising a stock of wild type *Drosophila melanogaster*, you isolate 2 interesting mutants: (1) a female with black eyes, and (2) a male that becomes sterile when incubated at 28 ºC (the control rearing temperature is 25 ºC).

In order to understand whether each of these phenotypes is due to a mutation in a single genetic locus, you decide to perform backcrosses with wild type flies.

These are the results you obtain:

For backcross (1): half of the flies have red eyes and half have black eyes. For both phenotypes, half of the progeny are males and half females.

For backcross (2): all the males become sterile at 28 ºC. All the females are fertile at all temperatures.

a) From those results, can you infer if the mutations you have isolated occurred in single genetic loci? Justify your answer.

To better understand the mutation that confers black eyes, you decide to cross mutant females with mutant male progeny from backcross 1.

This is the result you obtain:

All females have black eyes. Half of the males have black eyes and half have red eyes.

b) From those results, can you establish if the black eye phenotype is dominant or recessive? Is the causative mutation on an autosome or on a sex chromosome? Use a Punnet square to justify your answer.

c) What would have been the result of the cross of mutant female with mutant male progeny from backcross 1 if the mutation was dominant and on an autosome? Use a Punnet square to justify your answer.

d) From the results of backcross (2), can you infer if the mutation that causes sterility in males at 28 ºC is on an autosome, or on a sex chromosome? Can you say if the sterility phenotype is dominant or recessive?

8. Polymerase chain reaction (PCR)

Your experimental goal is to clone a gene. The sequence of the coding strand of the gene is shown below. The start codon in all the indicated sequences is emboldened.

One easy way of cloning is to PCR-amplify the DNA region of interest, incorporating restriction endonuclease sites for either *Bam*HI (see PRIMER 1 and PRIMER 2 below) or *Hind*III (see PRIMER 3 below). These restriction sites are underlined into each primer. That way, after the target DNA region has been amplified, the resulting "amplicon" can be digested with the appropriate restriction endonuclease(s). The digested product can then be ligated to a suitable recipient plasmid that has been cut with the same endonucleases. The cloned gene is located on the plasmid just downstream (and therefore, under the control of) a plasmid-borne promoter.

5'-GATCGGATCCAGGAGGTGACAT**ATG**AACATCGCG-3' PRIMER 1
5'-GATCGGATCCAGGAGGTGACAT**ATG**AACATCGCGCCACTCAAG-3' PRIMER 2

ATGAACATCGCGCCACTCAAGGTCTCTCTGCTCTCCCTCGCTCCTCTCCCCCGT
TGCCAGCCTCCCCGCCCATGCGGCCTGCACCGCCAACCTGGCCCTGCAACCC
GCCAGCAGCCTGCCCGCGCTCGGCGGGCGCCTGGTCTACCACAGCTATATGG
AGTACGGGGACGGCTCCTCGAACCTCTACCTGCACGATTTCAAGAGCAAGAGC
ACCCGCCAGCTCAACCAGCCCGGCTGGAACATCGAGGACCCGATGAATGCGC
ATTTCTCCCCGGACGGCCGCTACCTGACCTTCATGGGCCGCCAGAATGGCGCC
TGGCACGTGTTCGCCTGGGCCATCGGCGGTACCCAGGCGCCGAGCAACCTGA
CCGCGGCCATCGGCGGTCGCAACGAGGATCCGAAGTTCTCCTTCGACGGCCG
CCAGGTCGTGCTCAAGCACGAGGGCGACATCCGCCTCGCCACCCTGGTCTTCA
ATGGCGATGGCAGCGTCGGCGTGAGCGCCTGGAAAGCCGTGACCGGCGACG
GCTGGAGCACCGAGGAGTCGATGCCGTTCCTCACACCCTCAGGCAAGTACGTG
GTCTACGCGACAGGAGCCGGCGACAGCCTGCGGGTGGTGCGCAGGAACCTG
GAGAACGGCCAGGTCTGCGCCCTCGCCACCCCGGCCGCGGGGGGCCGCGAC
TATTACCCGGTGGTACGCGACTACACCGCCTACTTCCTCTCGCGTACCCAGCC
GGCCGGCAACGACCAGTTGGCCATGGTCGTACCCAACAGCCCGCCCGGCACC
CCGGCGATCCTGCCGCTCAACCACTGCCAGGGCGACAATTCCGACGCCGCGC
CGGTGAACGAGGACTACCTGATCTTCTCCAGCACTTCGTTCGACCCGACCTAC
AGCCTGCTCCTCGGCGACATCGCCGCGGCGCGGGTCTGGCGTCTCGACCCGG
CGCAGATCAACCTGCCCGACGGTCGGCAGAAGCTCGGCGCCAGCTATACCGC
GGCACGCTGA
3'-GATATGGCGCCGTGCGACTTTCGAAGATC-5' PRIMER 3

You decide to try two slightly different primers (PRIMER 1 and PRIMER 2) designed to anneal to the start of the open reading frame of the gene, but for the sake of economy, you use only a single primer (PRIMER 3) designed to anneal to the other end of the gene.

[Question continues on the next page]

A sample of genomic DNA, diluted to contain a single template molecule in each reaction mixture, is used as the PCR template. The PCR is carried out using either PRIMER 1 and PRIMER 3 ("P1, P3" in Figure 9), or PRIMER 2 and PRIMER 3 ("P2, P3" in Figure 9). After 35 cycles of amplification with an extremely sensitive DNA polymerase and no limitation on substrate availability, the reaction mixtures are resolved by agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 9 panel A. The predicted size of the amplicon is approximately 1,000 base pairs.

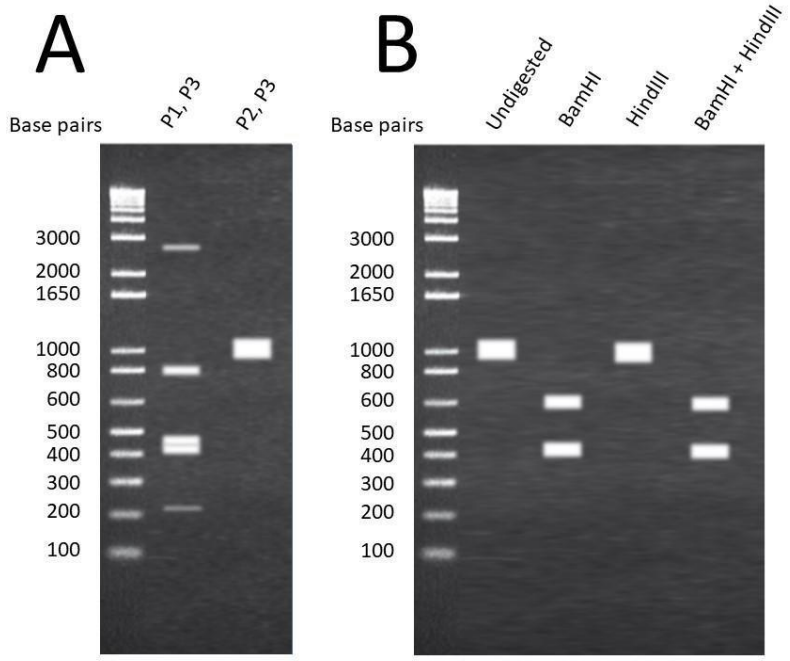

Figure 9. Gel electrophoresis analysis of PCR products. (A) The PCR products from amplification with either primer 1 and primer 3 (P1, P3), or with primer 2 and primer 3 (P2, P3) are shown. **(B)** Restriction digestion of PCR products from the P2+P3 reaction in A.

a) Briefly explain what a start codon is important for.

b) Look carefully at the sequence of PRIMER 1 and PRIMER 2. Between the *Bam*HI recognition site and the start codon is a sequence of bases. Given that you want to express the encoded open reading frame from a plasmid that encodes its own promoter, what do you think this sequence of primer-encoded bases might do?

c) Why is ethidium bromide added to the gel before electrophoresis?

d) How many grams of PCR amplicon are produced after 35 cycles of amplification? Avogadro's number is 6.02×10^{23}. Assume that the mean molecular mass of a base pair is 650 Da, and that the PCR product is 1,000 bp in length.

e) What do you conclude from the data in Figure 9A?

[Question continues on the next page]

The PCR product obtained using PRIMER 2 and PRIMER 3 was sequenced and found to be correct. The PCR product was subsequently purified and the pure DNA was treated with either *Bam*HI alone, *Hind*III alone, or a combination of *Bam*HI and *Hind*III. The resulting digests were resolved by agarose gel electrophoresis, as before. The results are shown in Figure 9B.

f) What do you conclude from the data in Figure 9B? You may assume that the restriction enzymes used are fully active in the reaction mixtures.

g) Which primer would you redesign, and how would you redesign it, in order to solve your problems?

h) Assuming that the open reading frame of the amplified gene (which encodes a protein known as “WD-40”) is 999 nucleotides in length, excluding the stop codon, and that the average molecular mass of an amino acid residue in a polypeptide is 110 Da, what is the molecular mass of the encoded protein?

i) You express and purify WD-40 and measure the molecular mass of the native, purified protein using biophysical approaches. The molecular mass obtained this way is 146 kDa. What do you conclude from this?

9. The embryological zoo

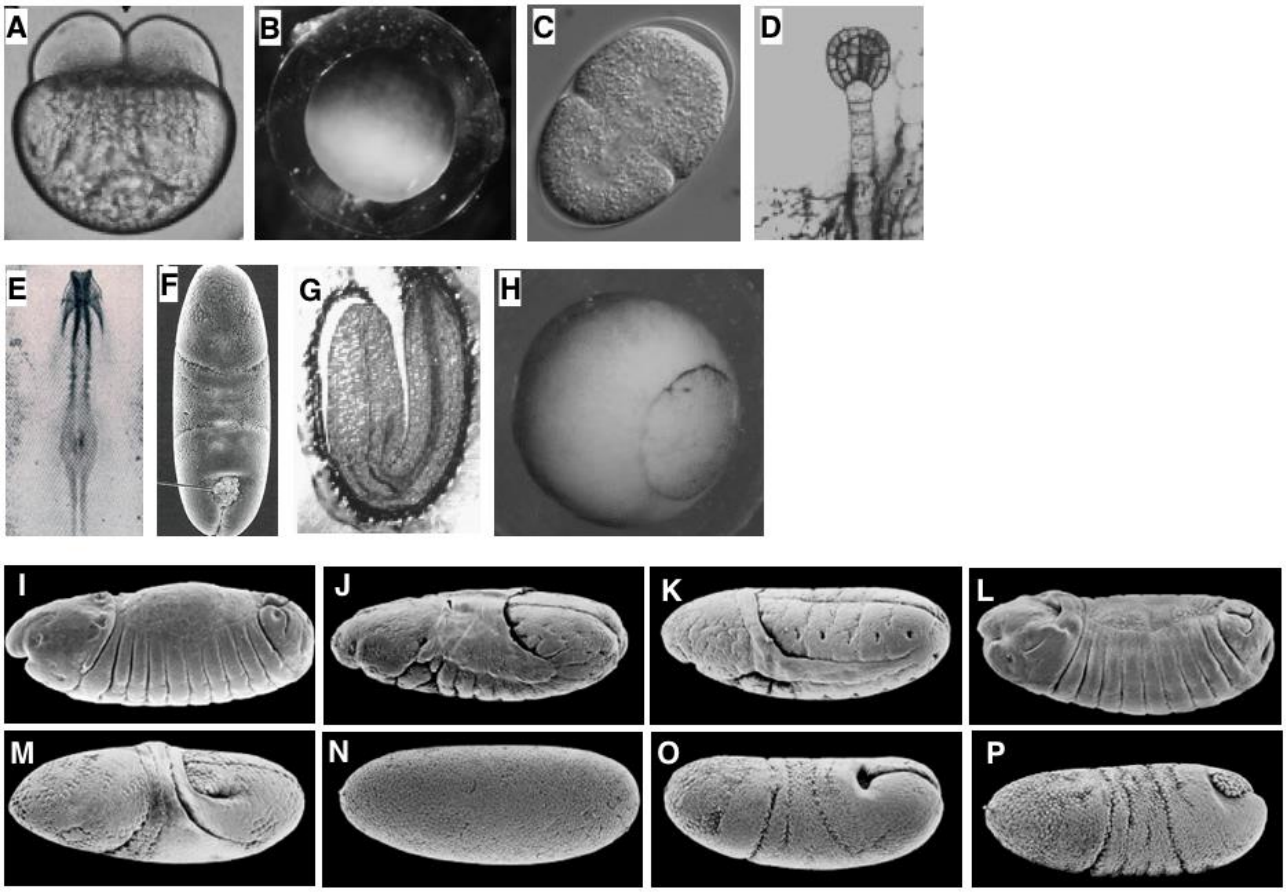

Figure 10. Micrographs of embryos from a range of species.

Figure 10, panels A-P show representative images of stages of embryonic development from different organisms.

a) When considering panels A-D, which image is the comparatively earliest in development regardless of the organism?

b) When considering panels A-D, which image is comparatively latest in development regardless of the organism?

c) When considering panels E-H, name the organism depicted in each panel (common or Latin names are acceptable).

d) When considering panels E-H, name and briefly describe the importance of one clear and defined structure in the developing embryo.

e) Panels I-P show representative images of stages of *Drosophila melanogaster* embryonic development. List the order of the panels from earliest to latest in development.

[Question continues on the next page]

f) Briefly discuss imaging advantages of each of the following for the study of embryogenesis: *Caenorhabditis elegans* (roundworm)*, Danio rerio* (zebrafish), and *Gallus gallus* (chicken)*.*

[END OF PAPER]