UNIVERSITY OF CAMBRIDGE

NATURAL SCIENCES TRIPOS PART IA

BOC/WP Biology of Cells – Practical

Thursday 10th June 2021

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*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.

Word process your answers in one of the following formats: .doc, .docx, .rtf or .txt (or with permission, hand write).

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.

All answers must be uploaded in one document which you should name in the following format:
Candidate number.format
For example, 7850X.pdf

Follow the guidance on the University Moodle site on how to upload your documents.

Candidates are permitted to use an approved calculator.

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. Bacterial plasmids
8. PCR
9. The embryological zoo

1. Microscopy

You are part of a team that studies the cellular organisation of Amoeba using microscopy. Lately, the population of Amoeba in Byron's pond has been decreasing significantly and you decide to take a sample of the pond water to investigate, using your microscope.

a) What are the parts labelled A B and C in Figure 1? Briefly describe their role.

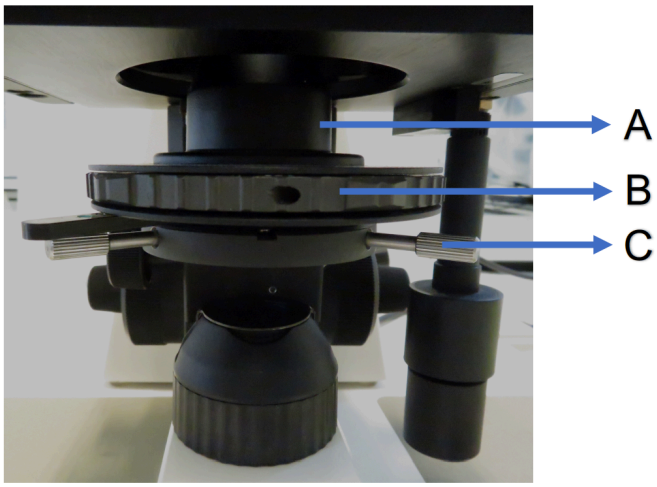


Figure 1. Microscope used to image Amoeba.

b) The sample of water you collected from Byron's pond has Amoeba, but also Chara. You start by setting up the microscope with the 10× lens. What adjustments would you make to components A and B (you may need to use other microscope components to set them), if you wanted to observe: i) Chara and ii) Amoeba?

c) While observing an Amoeba using phase contrast microscopy, you find organelles A and B (Figure 2). Identify the organelles A and B, and briefly describe the function of the structure labelled A.

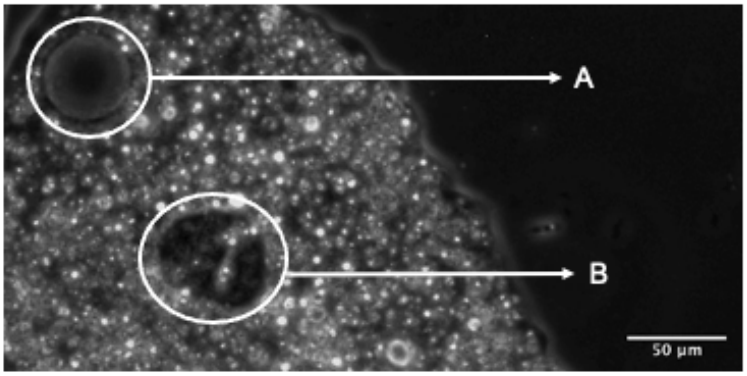


Figure 2. Phase contrast micrographs of Amoeba.

d) The Amoebae in your sample from the pond have difficulty catching prey. You decide to assess whether changes in the speed of Amoeba movement could explain this, and their declining numbers. Describe how, using the phase contrast microscopy, you can measure the speed of the Amoebae. Should you use any controls?

e) You observe that the Amoebae are moving slower than expected and you decide to investigate the mechanisms of cell movement. As Amoeba motion requires the motor protein myosin, suggest a microscopy-based approach to test whether the dynamic localization of myosin Amoebae from Byron's pond and wild type Amoebae are different from one another, using living cells.

f) You discover that the Amoeba are declining due to infection with a virus that is 50 nm in diameter. Discuss why phase contrast microscopy would be unsuitable to assay the presence of the virus in the Amoeba, and suggest an alternative microscopy technique and preparation method that would allow you to visualise the viruses.

2. Protein Structure

Aquaporins are integral membrane proteins that facilitate movement of water across phospholipid bilayers. The structure of an aquaporin is shown in Figure 3.

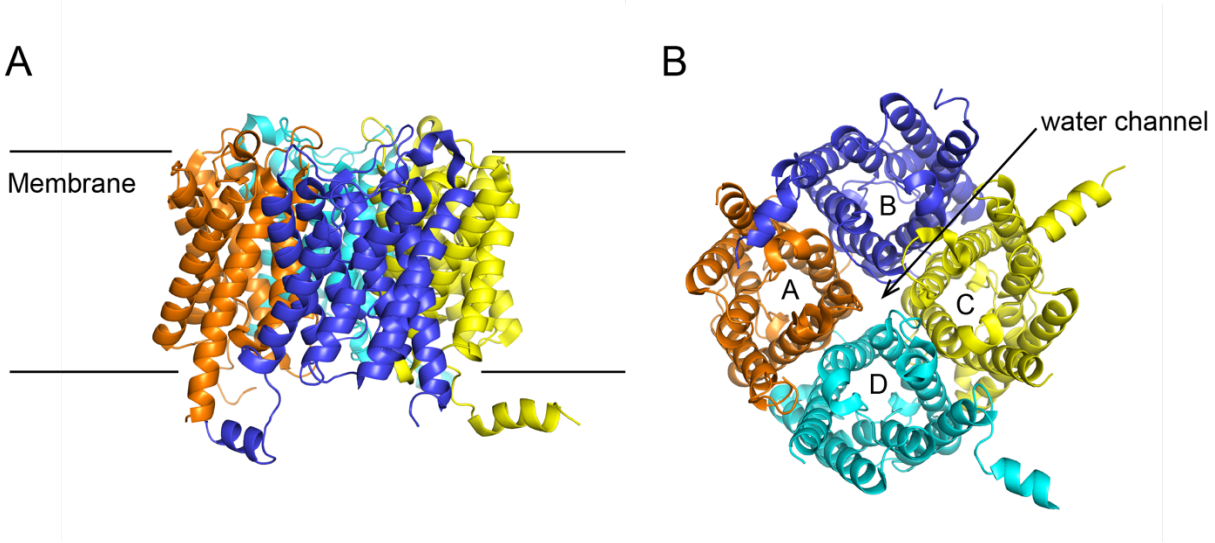


Figure 3. Structure of an aquaporin. (A) The structure of an aquaporin homotetramer shown in the plane of the membrane, and (B) viewed from the cytoplasm. The different monomer subunits are coloured light blue, dark blue, yellow and orange and labelled in B, where the location of the water channel is also labelled.

a) Which secondary structure elements are present in the aquaporin polypeptides?

b) Name 3 amino acids that you would expect to be located facing into the water channel formed by aquaporin. Justify your choices.

c) Name 3 amino acids that you would expect to be located on the surface of aquaporin that faces into the phospholipid bilayer. Justify your choices.

d) Sketch a Ramachandran plot for a single aquaporin monomer subunit, highlighting the region of the plot in which most bond angles would occur.

Figure 4 shows an ion channel, which is also an integral membrane protein.

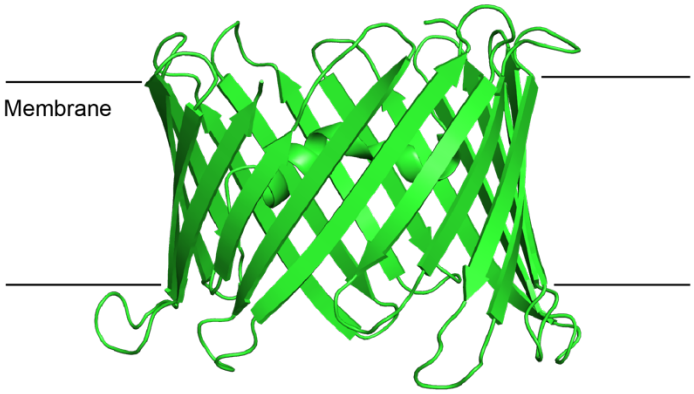


Figure 4. Structure of an ion channel shown in the plane of the membrane. The ion channel is shown in green and the lines indicate the position of the membrane.

e) Sketch a Ramachandran plot for this protein. What is the predominant secondary structure element seen in this protein?

3. Quantification of DNA and protein

You are working as a summer student in a virology laboratory and you have carefully extracted DNA and protein separately from four experimental samples, giving eight tubes in total.

However, 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	0.001	0.087	0.097	0.002	0.076	0.055	0

a) Calculate the concentration of DNA in the original eight samples in mg/ml. Which tubes do you think contained 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	0.15	0.30	0.42	0.51	0.59	0.63	0.65

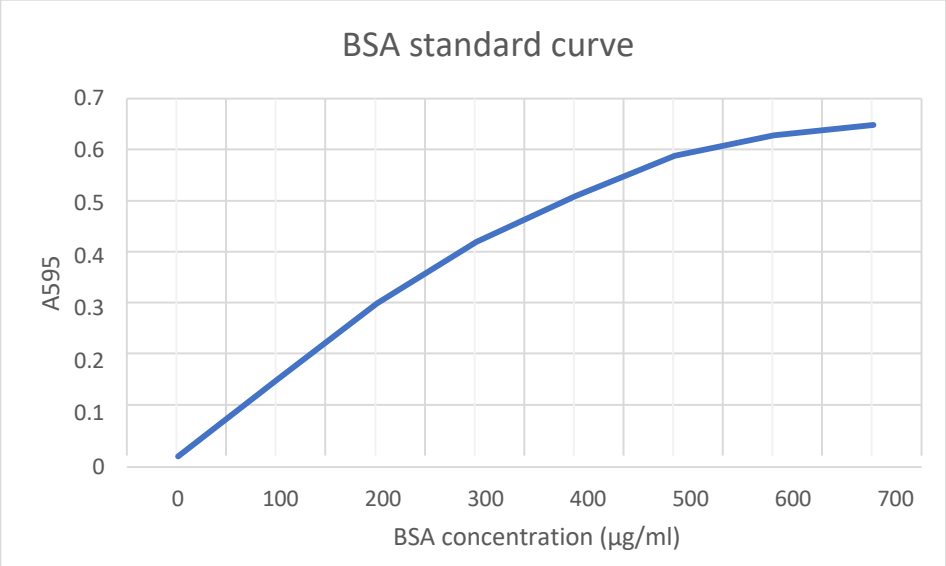


Figure 5. BSA standard curve A_{595} data. Data from Table 3 are plotted.

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}	0.60	0.61	0.01	0.02	0.40	0.00	0.01	0.52

b) Calculate the amount of protein (µg) in the 50 µl of the eight samples added to the Bradford reagent. Which tubes do you think contained the protein samples?

c) How does Bradford reagent work in this assay? What are the limitations of the reagent/assay and how does this relate to your measurements?

d) How could you perform experiments to resolve these limitations?

4. Enzyme activity

The positive strand RNA virus SARS CoV-2 encodes an RNA-dependent RNA polymerase (RdRp) to duplicate its genome.

Your colleagues in Oxford are studying SARS CoV-2 RdRp and have request your help, given your understanding of lysozyme enzyme kinetics.

To test the kinetics of this enzyme, purified RdRp is used to synthesize RNA in the presence of UTP and the 20 bp primed template shown below.

5'-GUCAUUCUCCUAAGAAGCUA-3'
3'-CAGUAAGAGGAUUCUUCGAUAAUUUUAGUGUA-5'

The initial rate of the reaction at different UTP concentrations is shown by the graph in Figure 6.

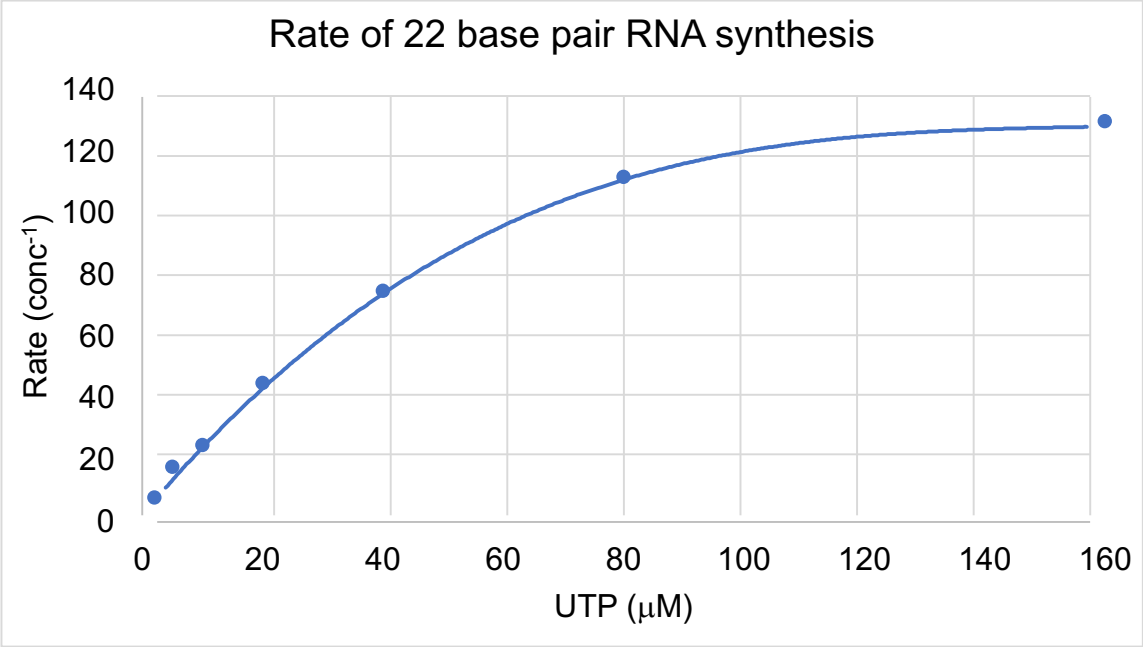


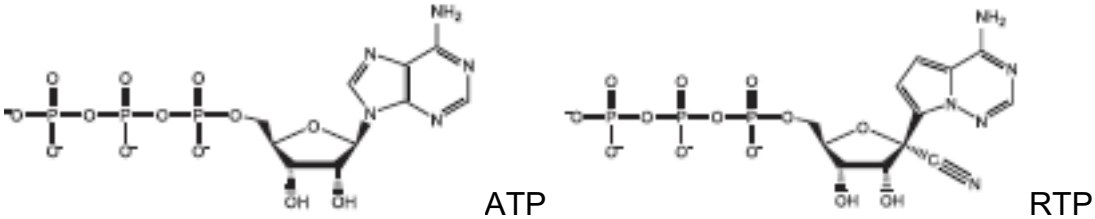
Figure 6: The rate of 22 bp RNA synthesis by SARS CoV-2 RdRp with varying UTP concentration.

a) From this graph the presumed K_m for this reaction is estimated to be 74 mM. What is the V_{max}?

b) Why is this reaction measuring a 22 bp RNA product?

c) What assumptions are being made to obtain the Michaelis-Menten constant? Do you think that Michaelis-Menten analysis is appropriate for this reaction?

The nucleotide RTP (Figure 7) acts as a competitive inhibitor of ATP for SARS CoV-2 RdRp. The drug remdisivir when ingested is a source of RTP and a therapy for Covid-19.



ATP RTP

Figure 7. The structure of ATP (left) and RTP (right).

The effectiveness of the triphosphate form of this inhibitor (RTP) in preventing catalysis by RdRp is determined by the ratio of incorporation of ATP versus RTP and is governed by the equation:

$$\text{Inhibitor incorporation} = (k_{cat}/K_m)_{ATP} / (k_{cat}/K_m)_{RTP}$$

In the same assay, described above, using the RdRp of SARS CoV-2, the 20 bp primed substrate + UTP, the following values were obtained for the additional incorporation of ATP or RTP (Table 4). For RTP, different values were obtained for the incorporation of the first, second and third RTP into the RNA.

Table 4. Efficiency of nucleotide incorporation by RdRp using ATP or RTP.

Nucleotide	kcat/Km (mM^{-1} s^{-1})	kcat (s^{-1})	Km (mM)
ATP	0.74	240.00	320.00
1st RTP	1.29	68.00	53.00
2nd RTP	0.19	3.62	19.00
3rd RTP	0.18	3.76	21.00

d) Which nucleotide triphosphate has the greatest affinity for SARS CoV-2 RdRp?

e) Why are the kinetics of the first and then the second RTP different?

5. Photosynthesis and the oxygen electrode

You discovered a new compound 'phytothan' that is a potent herbicide, which you hypothesize is targeting the chloroplast thylakoid electron transport chain.

To investigate this hypothesis, you decide to use an oxygen electrode and chloroplasts purified from Arabidopsis leaves.

You homogenize the Arabidopsis leaves in 5 ml of 0.4 M sucrose buffer. The homogenate was centrifuged at 3000 *g* and the chloroplast pellet was resuspended in 1 ml of sucrose buffer.

100 µl of the chloroplast suspension was removed and added to 900 µl of acetone. 1000 µl of this solution were 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 was 0.633.

a) Calculate the chlorophyll concentration in (mg^{chl} ml^{-1}) in the Arabidopsis leaf extracts.

200 µl of the resuspended chloroplasts were placed in an oxygen electrode containing potassium cyanide (KCN). The final volume in the electrode chamber was 1 ml. The change in oxygen tension in the electrode over a time-course was measured.

For the first 120 seconds the apparatus was kept in the dark. For the next 120 seconds the apparatus was exposed to light. For the final 120 seconds, the lights were on and phytothan was added.

The experiment was repeated twice; once with the addition of phenyl quinone, and once with the addition of ferredoxin.

Data from the experiment are shown in Table 5.

Table 5. Oxygen electrode measurements from Arabidopsis chloroplast preparations.

Light/Dark	Phytothan	Time	+Phenyl Quinone mV	+Ferredoxin mV
Dark	-	0	401	388
Dark	-	30	406	393
Dark	-	60	408	393
Dark	-	90	413	398
Dark	-	120	418	404
Light	-	150	450	430
Light	-	180	480	466
Light	-	210	510	498
Light	-	240	540	518
Light	+	270	575	552
Light	+	300	600	586
Light	+	330	628	614
Light	+	360	658	644

An electrode reading of 1 mV is equivalent to 2.55×10^{-4} O_2 µmoles ml^{-1}. The lights were turned on immediately after the dark measurement at 120 seconds. Phytothan was added immediately after the measurement at 240 seconds.

b) Calculate the photosynthetic rate (µmole O_2 min^{-1} mg^{chl-1}) in the presence of the phenyl quinone, (i) before and (ii) after addition of phytophan.

c) Calculate the photosynthetic rate (µmole O_2 min^{-1} mg^{chl-1}) in the presence of the ferredoxin, (i) before and (ii) after addition of phytophan.

d) Why is it necessary to add phenyl quinone or ferredoxin to the chloroplast suspension?

e) From these data, what can you conclude about the possible targets of phytophan within the thylakoid electron transport chain?

6. Fungal genetics

After random mutagenesis of a wild type *S. cerevisiae* strain, you select an interesting mutant that grows well at 23°C, but cannot grow at 37°C (temperature sensitive (ts) mutation).

You want to know if this phenotype is due to a mutation in a single gene, or in multiple genes.

a) Propose a simple experiment to distinguish between these 2 possibilities.

You conclude that the phenotype is due to a mutation in a single gene. The analysis of cells cultivated at 37°C reveals a uniform arrest as budded cells. Propose an experiment to test the following possibilities:

b) Cells arrest before DNA replication has started.

c) Cells arrest after DNA replication has been completed.

d) Cells arrest in anaphase.

The analysis reveals that the cells are arrested in anaphase. In the freezer of your lab you already have 3 ts mutants that arrest in anaphase: *tem1-3*, *dbf2-2* and *cdc15-1.*

e) How would you determine whether your mutant is an allele of TEM1, DBF2 or CDC15?

You want to genetically map the gene responsible for the ts phenotype, so you decide to cross it with a triple mutant strain. This triple mutant accumulates red pigment on medium with limiting amount of adenine (*ade2*), cannot grow on medium lacking uracil (*ura3*) and requires its growth medium to contain tryptophan (*trp1*). Note that your ts strain is ADE2, URA3 and TRP1 (i.e. wild type at these 3 loci).

You establish a diploid, put it on starvation conditions to induce meiosis and analyse 5,000 of the resulting haploid spores. The results you obtained are shown in Table 6.

Table 6. Results of the genetic cross. * indicates growth on medium with limiting amounts of adenine

White colonies*	Red colonies*	Ability to grow without uracil	Ability to grow without tryptophan	Ability to grow at 37°C	Number of spores analyzed
+		+	+	+	212
	+	+	+	+	196
+		+	+	-	700
	+	+	+	-	690
+		-	-	+	794
	+	-	-	+	776
+		-	-	-	72
	+	-	-	-	94
+		-	+	-	304
	+	-	+	-	320
+		+	-	+	344
	+	+	-	+	352
+		-	+	+	34
	+	-	+	+	42
+		+	-	-	34
	+	+	-	-	36

f) On the basis of these results, calculate the single trait ratio for each gene. Comment on the results you obtain.

g) Propose a genetic map for the genes you have analysed. Indicate clearly the number of chromosomes involved and the map distances between the genes.

7. Bacterial plasmids

An alarming number of students at Gonville Hall have been struck down by severe food poisoning. Efforts to find a cure have been hampered by the fact that the causative bacterium (*Escherichia caian*) is resistant to the antibiotics chloramphenicol, ampicillin and tetracycline. Describe how you would employ the techniques used in the Biology of Cells on-line practical to determine the following facts. In all parts of the question, use diagrams to illustrate the expected results of any electrophoretic analysis that you might carry out and explain how the gel results would be interpreted.

a) Whether the antibiotic resistance genes are chromosome- or plasmid-encoded?

b) Whether multiple resistance is due to one plasmid carrying several resistance genes, or to multiple plasmids each with a single gene?

A subsequent outbreak of food poisoning at St Joan's College is resistant to only two antibiotics: ampicillin and chloramphenicol.

c) Once again using only techniques from the Biology of Cells on-line practical, how would you test whether there was a plasmid in common between the two outbreaks?

Finally, the outbreak spreads from St Joan's to neighbouring Michaelhouse. Isolates from the Michaelhouse kitchens again yield a plasmid resistant to ampicillin and tetracycline but, on an agarose gel, it appears much larger than the plasmid from St Joan's.

d) Give a possible explanation for this observation.

8. PCR

You are tasked with PCR-amplifying a gene (*betR*) from a newly characterised Gram-negative bacterium, *Meliorremis oxfordensis*. Most of the *betR* open reading frame is located in the dotted region shown in Figure 8 (denoted “rest of gene”). The DNA sequence of the regions just upstream (to the left in Figure 8) and downstream (to the right in Figure 8) of the gene are as indicated.

You design three primers (primer 1, primer 2 and primer 3, respectively) to anneal upstream of *betR* and one primer (primer 4) to anneal downstream of *betR*. The sequence of each primer is shown in Figure 8. Following 30 cycles of PCR-based amplification with the primer combinations indicated in Figure 8, the amplified products were resolved by agarose gel electrophoresis in the presence of ethidium bromide. A “sizing ladder” was run alongside the PCR-amplified products, with relevant sizes (in base pairs) as indicated.

a) From inspection of the gel, estimate the approximate size of the DNA in each of the bands 1, 2 and 3.

b) Only band 1 is of the size predicted for the amplified *betR* gene. What is the likely origin of bands 2 and 3?

Figure 8. PCR analysis of (*betR*) from *Meliorremis oxfordensis*.

c) Calculate the approximate melting temperature of primer 1. Explain your reasoning.

d) The melting temperature for primer 3 and for primer 4 was calculated to be 58°C. What annealing temperature would you use in PCR reactions containing these primers? Briefly explain your reasoning.

e) How might you change the position of primer 1 to improve amplification?

f) An experienced colleague points out that primer 2 is not well-designed. They are correct. Why?

Band 1 from reaction mixture C was extracted from the agarose gel and primer 3 (shown in blue below in Figure 9) was used as a sequencing primer in a Sanger DNA sequencing reaction. The obtained sequence is shown in grey below in Figure 9.

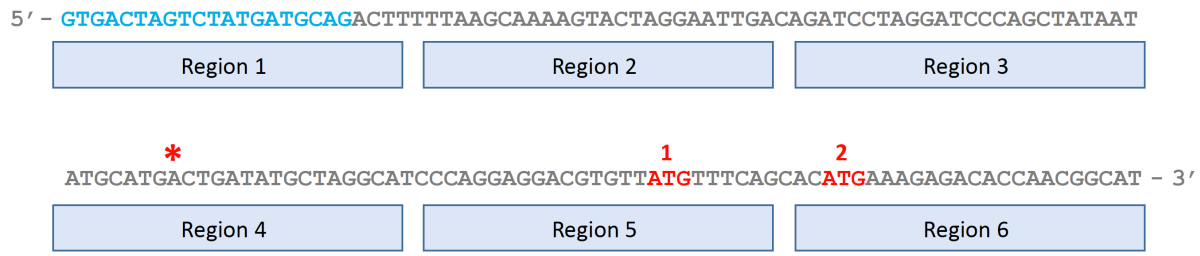


Figure 9. Results of Sanger DNA sequencing analysis.

g) Two potential translational start sites, 1 and 2 (indicated in red) were identified by computational analysis. Explain which of these predicted *betR* translational start sites is more likely to be correct?

h) The transcriptional start site of *betR* was determined to be the adenosine indicated by the asterisk. Write down the most likely sequences of the -10 and -35 promoter elements of *betR*.

i) Write down the sequence of the likely start codon in *betR*.

j) BetK is a transcription factor known to bind to the upstream region of *betR* and physically block the binding of RNA polymerase to the promoter region. Which of the indicated regions is likely to contain the BetK binding site? Briefly explain your reasoning.

9. The embryological zoo

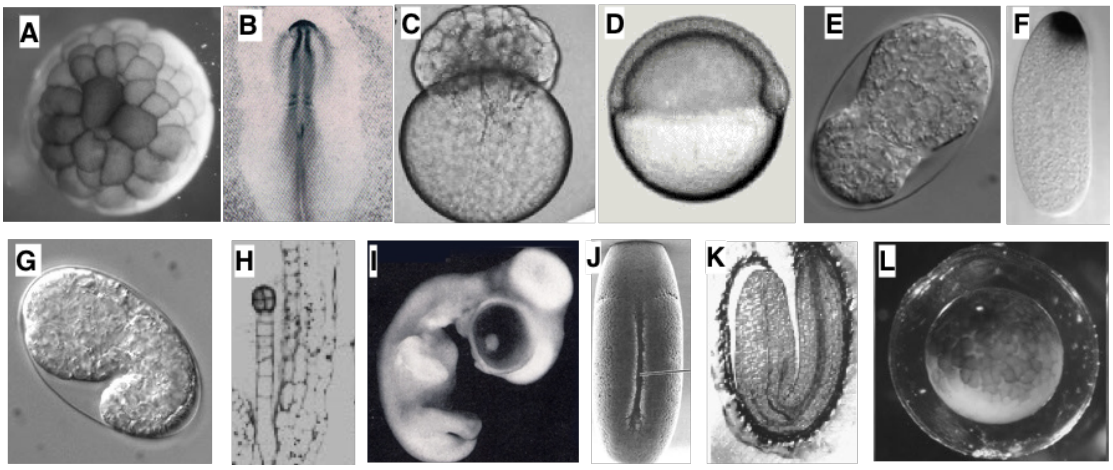


Figure 10. Stages of embryonic development in six different species. There are two images for each species at different stages of development.

a) Panels A-L of Figure 10 show representative images of stages of embryonic development from six different organisms. Name the organism depicted in each panel (common or Latin names are acceptable).

b) When considering panels A-L, for each of the six organisms, which of the two images shows the earlier stage of development?

c) What is the outcome of gastrulation in *Xenopus laevis* (frogs)?

d) Name two aspects of development that the organiser in *Xenopus laevis* is responsible for?

e) *Gallus gallus* (chick) is often used as a model organism. Give two advantage of *Gallus gallus* as a model organism.

f) *Arabidopsis thaliana* (plant) is often used as a model organism. Give one advantage of *Arabidopsis thaliana* as a model organism.

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