UNIVERSITY OF CAMBRIDGE

NST0: NATURAL SCIENCES TRIPOS Part 1A

Saturday 10 June 2023 9 to 12

BOC/WP: BIOLOGY OF CELLS – PRACTICAL

Answer **all** questions.

All questions carry equal marks.

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

Diagrams or other handwritten material:

Where appropriate (e.g., for formulae, equations or diagrams) you may hand write your answers using the Inspera Scan paper provided.

Candidates should not copy and paste images from any other documents.

Note:

- Inspera Scan question codes **MUST** be noted during the exam.
- Please ensure that each Inspera Scan page is labelled appropriately, including your Blind Grade Number.
- Inspera Scan pages **MUST** be placed in the Inspera Scan folder at the completion of the exam and left on your desk.
- Inspera Scan pages will be scanned by administrators after the completion of the exam.

Stationery requirements:

- Rough work pad
- Inspera Scan pages
- Inspera Scan folder

SPECIAL REQUIREMENTS

Approved calculators
A ruler

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

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

1. Quantifying Protein and Nucleic Acids
2. Protein Structure
3. Enzyme Activity
4. Photosynthesis and the Oxygen Electrode
5. Fungal Genetics
6. Bacterial Plasmids
7. PCR
8. Imaging Cell Division and Microscopy
9. The Embryological Zoo

1. Quantifying Protein and Nucleic Acids

You have extracted RNA from leaves, stems and roots of a plant and want to quantify the amount of RNA in each sample using a spectrophotometer to measure the absorbance at 260 nm. However, the values you measure are much higher than expected. You wonder whether something is wrong with the spectrophotometer. Luckily, a colleague in the lab has a set of RNA samples with known concentrations that they previously measured with a different machine.

You want to use these samples to test whether you get the expected results using your spectrophotometer. You start to calculate the expected absorbance values for the samples of known concentration. The results are shown in Table 1.

Table 1: Expected absorbance values.

Tube	**1**	**2**	**3**	**4**	**5**
Concentration (µg/mL)	0	750	1250	1800	2300
Absorbance at 260 nm	0	0.01875	0.03125		

Assume that the extinction coefficient for RNA at 260 nm is 25 litres cm^{-1} g^{-1}. The path length of the cuvette you are using is 1 cm.

a) Complete Table 1 by calculating the expected absorbance values for samples 4 and 5. Show your workings.

You then measure the absorbance values of the known samples on your spectrophotometer. The values are shown in Table 2.

[Question continues on the next page]

Table 2: Absorbance values of the known samples.

Tube	**1**	**2**	**3**	**4**	**5**
Concentration (µg/mL)	0	750	1250	1800	2300
Absorbance (at 260 nm)	0.001	0.019	0.030	0.045	0.056

b) Assuming that the acceptable error range for your measurements is ± 5%, would you use the spectrophotometer for future measurements? Provide a brief justification for your answer.

You also want to measure how much protein there is in your leaf and root samples. For this, you use the Bradford assay and set up a standard curve using samples of known BSA (Bovine Serum Albumin) concentrations. The results are given in Table 3.

Table 3: BSA concentrations and absorbance values

Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
BSA concentration (µg/mL)	0	100	200	300	400	500	600	700	800	900
Absorbance (at 595 nm)	0.01	0.11	0.22	0.34	0.43	0.50	0.55	0.58	0.60	0.61

c) Plot the BSA standard curve on graph paper.

d) The absorbance of the leaf and root samples were measured as A_{595} = 0.15 and A_{595} = 0.59 respectively. Calculate the protein concentrations of the samples.

e) Do these results represent the true concentrations of protein in the leaf and root samples? Briefly explain why.

[Question continues on the next page]

f) What could you do to improve the reliability for any reading you are unsure of?

Another researcher in your group uses a different method to determine protein concentrations and gets higher readings for both samples.

g) Give one reason why the Bradford assay might underestimate protein concentration.

2. Protein Structure

The Ramachandran plot for a protein is shown in Figure 1:

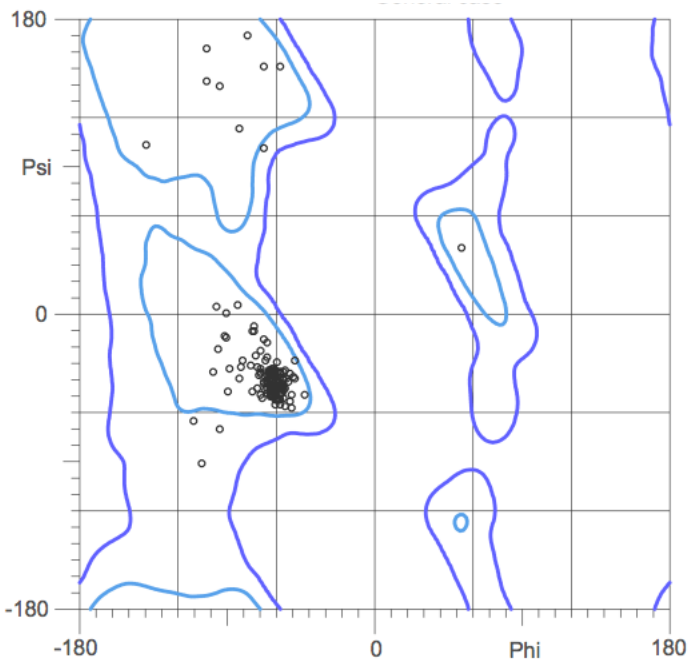

Figure 1: Ramachandran plot for a protein.

a) There are 248 amino acids in this protein, what secondary structure do most of these amino acids reside in? Explain your reasoning with reference to Figure 1.

The protein is a bacterial proton transporter and assembles into a barrel shape, see Figure 2.

[Question continues on the next page]

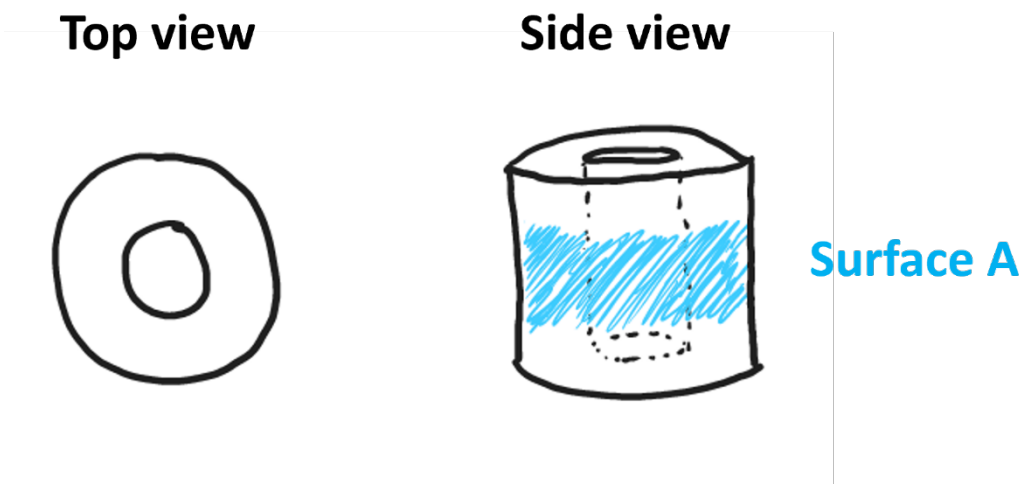

Figure 2: Sketch of the bacterial proton transporter.

b) Two amino acid residues in surface A (the shaded area in Figure 2) are valine and phenylalanine. Where is this protein most likely to be located in the bacterial cell?

c) Give 3 examples of other amino acid residues that are likely to be found on surface A and explain why.

d) This protein can change conformation depending on pH. Name 2 amino acids with R groups that have different chemical properties in acid versus alkaline conditions.

e) In order to activate proton transport, some parts of this protein move away from each other. Explain why you might find glycine residues in the linker between these mobile regions?

3. Enzyme Activity

In a practical class, students were provided with a liquid culture of the Gram-positive bacterium *Micrococcus luteus* and a solution of lysozyme from hen egg white, in order to investigate the effect of lysozyme on *M. luteus*.

You determine the effect of lysozyme on the *M. luteus* culture by measuring the OD_{600} of the culture using a spectrophotometer. Your results are shown in Figure 3.

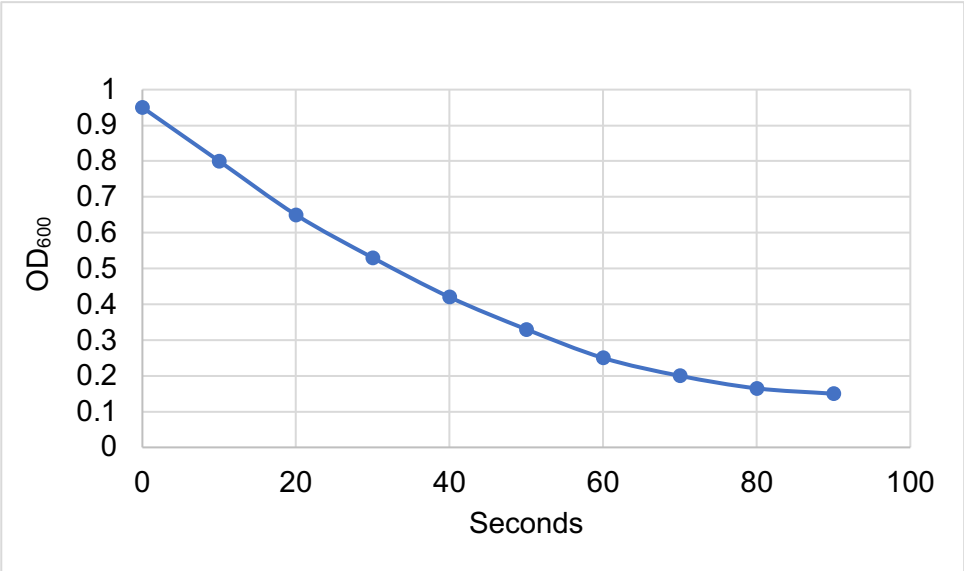

Figure 3: Change in OD_{600} of *M. luteus* culture treated with lysozyme.

a) How and why does the addition of lysozyme affect the OD_{600} measurement of the *M. luteus* culture?

b) Why is it important to measure lysozyme activity with a Gram-positive bacterium?

c) Why does the graph become non-linear very rapidly in this reaction? Note that the substrate is not limiting and lysozyme remains active throughout.

d) Calculate the rate of this reaction, measured in change in OD per second.

[Question continues on the next page]

Lysozyme cleaves sugars made up of repeating N-acetyl glucosamine (NAG) subunits or repeating NAM (N-acetyl muramate)-NAG subunits. The active site of lysozyme can accommodate up to six sugar subunits such as:

i) NAG-NAG-NAG-NAG-NAG-NAG
ii) NAG-NAM-NAG-NAM-NAG-NAM

The binding of the 4th sugar subunit, which is at the catalytic site of lysozyme, is energetically unfavourable, suggesting that this subunit must be conformationally altered to fit within the catalytic site. Oligomers of NAG made up of 4 or less subunits act as inhibitors of lysozyme.

e) How can lysozyme catalyse the cleavage of these sugars if the binding of the 4th sugar subunit is energetically unfavourable?

f) Why are sugar oligomers of 4 subunits or smaller lysozyme inhibitors?

g) Why do you think lysozyme can cleave sugars made up from alternating NAG-NAM subunits but not sugars only made from NAM, or even NAM-NAM-NAM-NAM-NAG-NAG?

Two acidic amino acid residues in lysozyme's active site (glutamic acid 35 and aspartic acid 52) are critical for the hydrolysis of peptidoglycan. The pK value for the carboxyl group side-chain of glutamic acid 35 is pH 6.7, while the pK value of aspartic acid 52 is pH 3.8.

h) pH 5-6 is the optimum pH range for lysozyme's hydrolytic activity. Are glutamic acid 35 and aspartic acid 52 protonated or deprotonated at this pH?

[Question continues on the next page]

i) An intermediate in the hydrolysis reaction is a positively charged oxonium ion in the NAM sugar subunit. Which of glutamic acid 35 and aspartic acid 52 is the proton donor to make the oxonium ion and which stabilises the positively charged intermediate?

j) Is stabilisation of the oxonium ion by an acidic side chain residue in the active site of lysozyme an example of a hydrogen bond, a van der Waals interaction or an electrostatic interaction?

4. Photosynthesis and the Oxygen Electrode

You have grown Arabidopsis in two different conditions, low light and high light.

You harvest 8 grams of seedlings grown under either low or high light and homogenize them in 30 mL of sucrose buffer. The homogenate is then centrifuged at 3000 g and the chloroplast pellet is resuspended in 4.5 mL of sucrose buffer and kept on ice.

400 µL of the chloroplast suspensions are removed and added to 900 µL of acetone. 1000 µL of this is used to measure the 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 the low light sample was 0.51 and the measurement from the high light sample was 0.80.

a) Calculate the amount of chlorophyll (mg chl) in the 4.5 mL sucrose resuspension for the low and high light samples. Show your workings.

b) Would you have expected these chlorophyll amounts in the two samples, considering the light conditions under which the plants were grown? Explain why.

c) Why is acetone added to measure the amount of chlorophyll?

20 µL of the resuspended chloroplasts from the high light sample were placed in an oxygen electrode containing KCN and one of the two electron acceptors, ferricyanide or phenyl quinone. The final volume in the electrode chamber was 1 mL. The change in oxygen tension in the electrode over a time-course was measured. Lights were turned on immediately after the dark measurement at 120 seconds. An electrode reading of 1 mV is equivalent to 2.55×10^{-4} µmoles O_2 mL^{-1}.

[Question continues on the next page]

Table 4 shows measurements in the presence of phenyl quinone. Similar readings were obtained in the dark and light with ferricyanide as an electron acceptor.

Table 4: Oxygen electrode measurements with the electron acceptor phenyl quinone.

Light/Dark	**Time (s)**	**Oxygen tension (mV)**
Dark	0	402
Dark	30	408
Dark	60	411
Dark	90	416
Dark	120	422
Light	150	548
Light	180	671
Light	210	795
Light	240	924

d) Calculate the photosynthetic rate of chloroplasts in µmole O_2 min^{-1} mg_{chl}^{-1} in the presence of the electron acceptor phenyl quinone. Show your workings.

You add a herbicide to the chloroplast solution that is known to inhibit photosystem I and measure oxygen evolution again using the two different electron acceptors.

e) With which electron acceptor would you expect to see a difference compared to chloroplasts that were not treated with the herbicide? Explain your reasoning.

f) Sketch a diagram that shows what you would expect to see for dark and light measurements for chloroplasts with the herbicide and in the presence of (i) phenyl quinone and (ii) ferricyanide.

5: Analysis of a Genetic Cross

You join a cell cycle lab that uses *S. cerevisiae* as a model organism. You are asked to follow up an observation made previously. The lab suspects the gene *TOM1* (for Termination Of Mitosis 1) is involved in completion of mitosis. Although a Δ*tom1* strain behaved indistinguishably from the parental WT strain, Δ*tom1* was synthetic lethal with a *cdc15ts* mutation (*CDC15* encodes an essential kinase required for the anaphase to telophase transition). Your supervisor asks you to confirm the observation. You are given a strain labelled "MAT**a**, *ura3-53*, *leu2Δ1* and *ade2-2* in which *TOM1* has been deleted using an *ADE2* cassette".

First, you confirm the strain's nutritional requirements using a replica-plating scheme. As expected, you find that the strain is unable to grow on plates lacking uracil or leucine. Against expectations however, the cells are unable to grow on plates lacking adenine but form white colonies when grown on medium containing a limiting amount of adenine.

a) Why is this observation surprising and how would you interpret it? How would you test your hypothesis (using techniques used in the practical)?

To test the involvement of *TOM1* in mitosis, you cross your Δ*tom1* strain with a yeast strain containing a *cdc20ts* allele that arrests the cell cycle in metaphase when incubated at 37°C.

The idea is to test the proposed synthetic lethality of Δ*tom1* with a ts allele of a gene involved in mitosis. The strain genotype is: *MATα, cdc20-1, ade2-2:* the strain can grow on medium lacking leucine or uracil but requires adenine and will form red colonies under limiting adenine. In addition, the strain is dead at 37°C with cells arrested in metaphase.

The phenotypes scored in ~890 progeny spores from the cross are presented in Table 5.

[Question continues on the next page]

Table 5: Distribution of phenotypic classes from the genetic cross

Ability to grow without uracil	**Ability to grow without leucine**	**White colonies on limited adenine**	**Ability to grow at 37 °C**	**Number of spores**
yes	yes	yes	yes	27
yes	no	yes	yes	140
yes	yes	no	yes	23
yes	yes	yes	no	90
no	yes	yes	yes	15
no	no	yes	yes	40
no	yes	no	yes	22
no	yes	yes	no	50
no	no	no	yes	184
no	yes	no	no	130
no	no	yes	no	25
no	no	no	no	5
yes	yes	no	no	30
yes	no	yes	no	40
yes	no	no	yes	11
yes	no	no	no	60

b) From these data, determine which genes are linked. Calculate the genetic distance between the linked genes (expressed in cM).

These data also show that *tom1*Δ and *cdc20ts* are NOT synthetic lethal.

c) Justify how this conclusion was reached.

[Question continues on the next page]

d) Which classes of spores would have been affected if the two mutations were synthetic lethal?

e) Does this result exclude the possibility that the Tom1 protein is involved in termination of mitosis? Justify your answer.

6: Bacterial Plasmids

The plasmids for the Biology of Cells practical were unfortunately lost during the 2020-21 phase of the Covid-19 pandemic. However, in the depths of the freezers in the Department of Genetics, various vials have been found from previous 1A students' work, along with some DH5α *E. coli* (which are plasmid-free). An attempt is made to transform the plasmid mixture into the *E. coli* cells following the BoC practical methodology.

a) What is the effect of treating the *E. coli* with ice-cold calcium chloride before adding the plasmid DNA mix?

The transformed cells are spread on agar plates containing either ampicillin (Amp) or chloramphenicol (Cam) and individual colonies are picked for further study. Three phenotypes are identified among the transformant colonies: AmpR CamR (resistant to both antibiotics), AmpR CamS (resistant to ampicillin alone) and AmpR CamS with a black-brown colour.

b) Based on your experience in the BoC practical laboratory, what might be the source of the dark-colour phenotype in some of the transformants?

c) Describe a control that you would include to ensure that drug resistance is due to the acquisition of a plasmid and not due to contamination.

Plasmid DNA was prepared from cultures derived from single colonies and analysed by agarose gel electrophoresis. This revealed the presence of three plasmids, one of 5.9 kb, one of 3.7 kb and one of 2.7 kb.

d) Describe briefly, with the aid of a diagram, how the size of a plasmid can be estimated from an agarose gel.

[Question continues on the next page]

The experimenters were surprised to find a plasmid of 7.4 kb in a very small number of colonies.

e) Give a simple explanation for this observation and how your hypothesis could be tested.

7. PCR

Figure 4 shows the time-temperature profile of a thermocycler during a PCR (polymerase chain reaction). The DNA polymerase used is Taq.

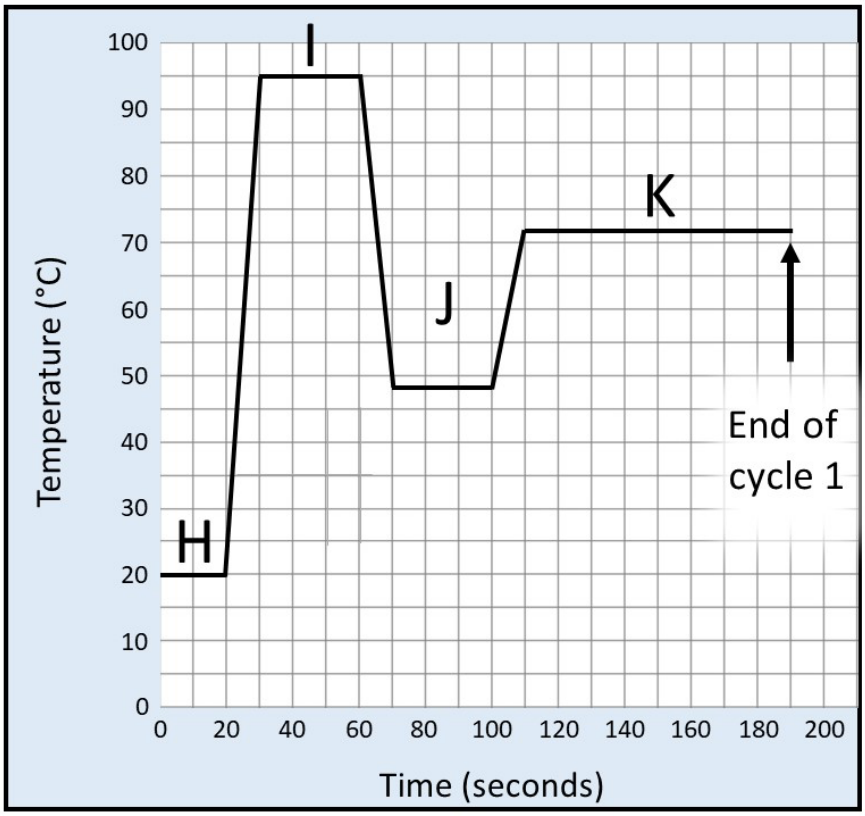

Figure 4: The time-temperature profile of a thermocycler during a PCR.

Consider the statements U – Z below:

U : Primers bind to the template DNA

V : Thermostable DNA helicase unwinds the template strands

W: Dimethylsulfoxide (DMSO) increases the stability of the primer-template interaction

X : Taq DNA polymerase unfolds, becoming inactive

Y : The template strands are thermally separated

Z : New DNA is synthesized by Taq DNA polymerase

[Question continues on the next page]

a) Which of the statements U – Z, if any, corresponds best with the individual steps H, I, J or K shown in Figure 4.

b) Assume that Taq DNA polymerase can synthesize exactly 1000 bases of DNA per minute. To the nearest whole number, what is the size (in bp) of the longest DNA fragment being amplified in the PCR shown in Figure 4?

c) For the PCR in Figure 4, the reaction sequence is repeated for 32 cycles. Assuming no limitation on substrate availability, and starting with 0.01 picogram of template DNA (1 picogram = 1×10^{-12} g), how many grams of product will be generated after 32 cycles?

d) Which of the primers (primers 1-4) below are best suited to be used in the thermocycling conditions shown in Figure 4? Briefly explain why.

5'-GATGCAACTGCTAGATCGATG-3'	Primer 1
5'-CAGACAGACTGATTGTC-3'	Primer 2
5'-CATGACCTAGACGTCCATGAC-3'	Primer 3
5'-GATCTGATGTCTATGTAC-3'	Primer 4

e) What is the maximum number of molecules of product that can be generated in the reaction? The reaction mixture contains 0.1 μM of the forward and reverse primers. Your total PCR reaction volume is 50 μL. Assume that (i) each primer is incorporated correctly during the PCR, (ii) no other reagents are limiting and (iii) Avogadro's number = 6.02×10^{23}.

[Question continues on the next page]

You are asked to design primers to PCR-amplify the region around an open reading frame (ORF) from a newly discovered bacterium. Your demonstrator says that the primer that you have designed to anneal to the 3' end of the ORF is fine. However, of the four primers you have designed to anneal near the 5' end of the ORF only one is appropriate. These four primers (primers 5-8) are shown below:

5'-CAGGAGGTCATGAATGA-3'	Primer 5
5'-GTCTATCGACAGTCATCG-3'	Primer 6
5'-CTTGACATATAATGCTTC-3'	Primer 7
5'-GGGGGTCTATGTACCCCC-3'	Primer 8

f) Which one of primers 5-8 would be most suitable for use in the PCR? Why are the other three primers less suitable?

8: Imaging Cell Division and Microscopy

Figure 5 shows selected frames from a time-lapse series of an animal cell during the mitotic cycle. Panels are shown in a random order.

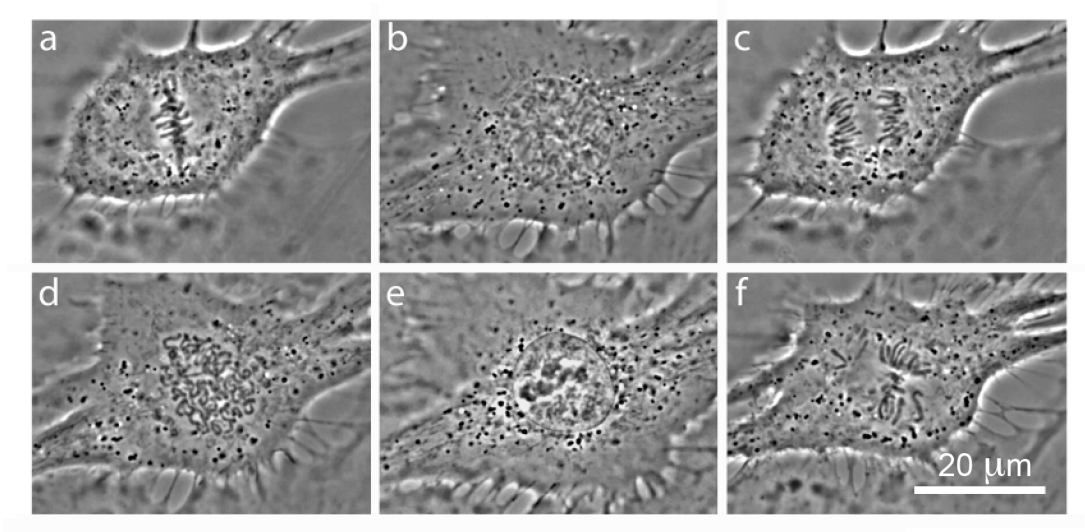

Figure 5: Microscopy images of a dividing cell.

a) What is the microscopy technique used? What are its advantages and limitations?

b) List the key adjustments you would make to the condenser iris and turret disc to collect the images shown in Figure 5.

c) Estimate the size of the nucleus in panel e.

d) Name the stage of mitosis represented and state the key cytological events, in each panel. In which order would the images appear in a time lapse series?

e) The cells also express yellow fluorescent protein fused to alpha-tubulin allowing you to view microtubules. What microscopy technique would you employ to image the samples? Draw the structure you would expect to see, relative to the images in Figure 5a and c.

[Question continues on the next page]

f) If cells were also expressing a cyan fluorescent protein fused to Mad2, describe what you would see in the stages depicted in panel (f) versus (a). Justify your answer.

9: The Embryological Zoo

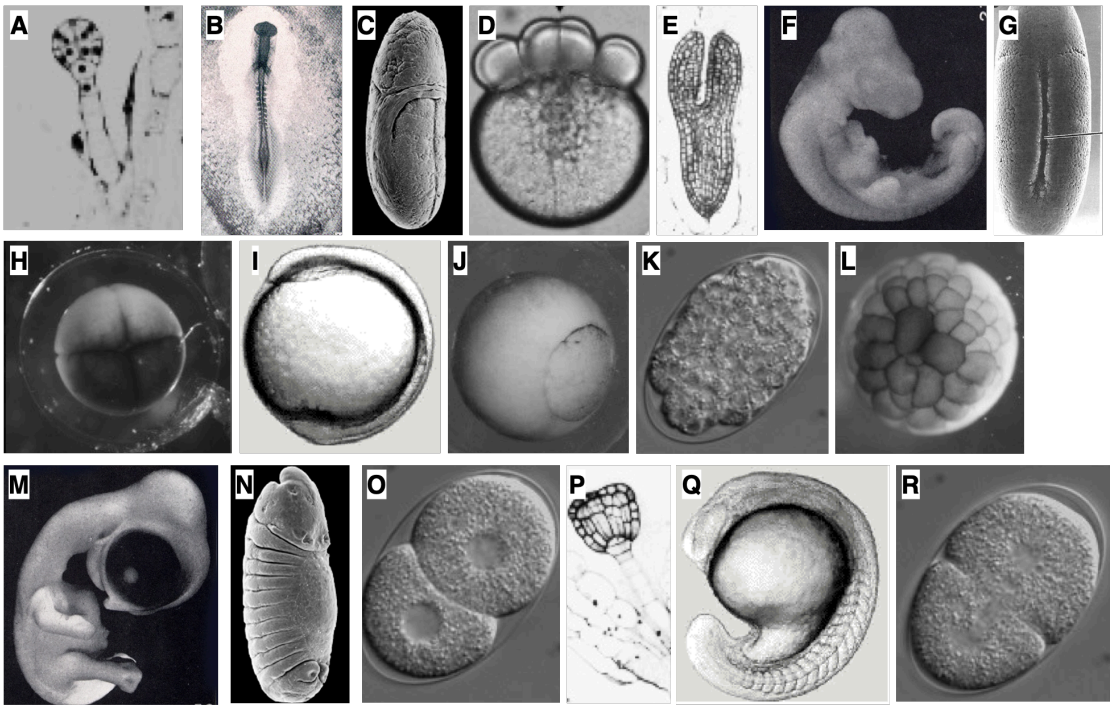

Figure 6. Stages of embryonic development in different species.

Panels A-R in Figure 6 show representative images of stages of embryonic development from six different model organisms.

a) Name all six organisms and list the panels from each organism in order from earliest in development to latest. When naming the organisms, common or Latin names are acceptable.

b) For each of the six organisms, give two characteristics that make them useful as a model organism.

c) Briefly describe how segmentation is different between flies and chicks.