UNIVERSITY OF CAMBRIDGE

NST0: NATURAL SCIENCES TRIPOS Part 1A

Saturday 8 June 2024 9.00 to 12.00

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 or draw on the Inspera Scan paper provided.

Candidates should not copy and paste figures or content from any other documents.

Note:

- Inspera Scan question codes **MUST** be noted during the exam.
- Please ensure each Inspera Scan page is labelled appropriately, including your Blind Grade Number.
- Inspera Scan pages **MUST** be places 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 to 20 minutes.

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

1(a) Microscopy

You are given the specimens listed below (i-vi) and asked to obtain images that will provide answers to the experimental questions that follow. For each specimen state which microscopical technique you could use:

(i) Cells of the giant algae, *Chara*, to determine the speed of cytoplasmic streaming.

Fill in your answer here

(ii) Mammalian hepatocytes, to estimate the width of the lumen of the rough endoplasmic reticulum.

Fill in your answer here

(iii) Rat pancreatic cells, to assess the distribution of nucleic acids.

Fill in your answer here

(iv) *Chara* cells, to study the localisation of the protein myosin fused to green fluorescent protein.

Fill in your answer here

(v) Living buccal epithelial cells, to estimate the number of cells in a sample of saliva.

Fill in your answer here

(vi) An unknown virus, to determine its morphology.

Fill in your answer here

Maximum marks: 3

1(b)

Briefly describe how the *Chara* samples in (i) and hepatocyte samples in (ii) would be prepared for the respective microscopy techniques.

(i) *Chara* samples

(ii) hepatocyte samples

Maximum marks: 4

1(c) When would you use scanning electron microscopy and when would you use transmission electron microscopy to analyse a sample? For each method give an example of a specimen that could be examined.

Fill in your answer here

Maximum marks: 3

2 Quantifying DNA and Protein Data Tables

Table 1: DNA absorbance measurements.

Tube	**1**	**2**	**3**
Absorbance (at 260 nm)	0.054	0.012	0.030
Concentration (mg/mL)			

Table 2: BSA standard curve absorbance measurements.

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

2(a) Quantifying DNA and Protein

You are a summer student working with *Arabidopsis* plants and have extracted DNA from three different leaf samples using a protocol that you received from another lab member.

a) You want to determine the DNA concentration in your samples using a spectrophotometer. For this, you add 10 µL of your sample to 990 µL water and measure the absorbance at 260 nm using a cuvette with a path length of 1 cm. The extinction coefficient for DNA is 20 litres cm^{-1} g^{-1}. Calculate the DNA concentrations in your samples given the absorbance values in **Table 1**, showing your workings.

Fill in your answer here

The protocol you used for DNA extraction looks different from other protocols you have used for this before. You are worried that your samples might be contaminated with substantial amounts of protein. You use a Bradford assay to test whether this is true. You first make a standard curve using a dilution series of bovine serum albumin (BSA) in phosphate-buffered saline (PBS) buffer. For each BSA sample, you mix 10 µL of the sample with 990 µL of Bradford reagent and measure the absorbance at 595 nm. The results are given in **Table 2**.

Maximum marks: 3

2(b) What solution would you use to calibrate your spectrophotometer before measuring each BSA sample? Why?

Fill in your answer here

Maximum marks: 1

2(c) You mix 10 µL of your DNA samples with 990 µL of Bradford reagent and measure the absorbance at 595 nm. Assume that for one of your samples, you obtained an A_{595} value of 0.27. Plot a standard curve with the BSA values given in Table 2 using the graph paper and calculate the protein concentration of this sample, expressed in mg/mL. Show your workings.

Use the Inspera paper provided or fill in your workings here.

Maximum marks: 4

2(d) You want to know how much protein is present in an *Arabidopsis* leaf. Describe how you would extract the total soluble protein.

Fill in your answer here

Maximum marks: 2

3 3) Enzyme Activity Table and Figure

Table 3: Initial rates of carbonic anhydrase reaction measured at different substrate concentrations.

CO_2 concentration (mM)	Initial rate (mM sec^{-1})
0	0
2	0.44
2.5	0.52
3	0.62
5	0.9
10	1.4
15	1.7
25	2.1

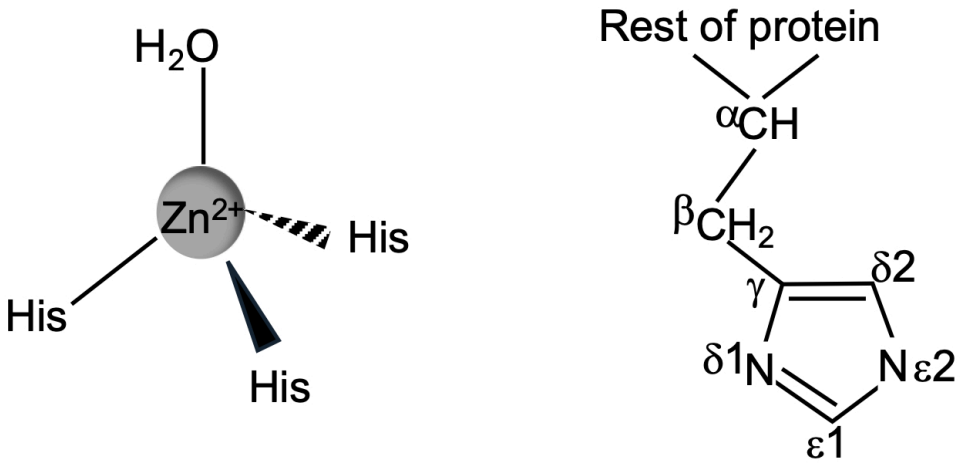
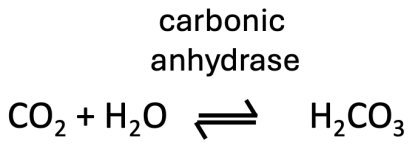


Figure 1: Coordination of Zn^{2+} ion in the active site of carbonic anhydrase (left) and a histidine side chain (right).

3(a) Enzyme Activity

Carbonic anhydrase catalyses the following reaction, which helps to transport carbon dioxide in the blood from respiring tissues:



As well as CO_2 transport, the product of the reaction helps to buffer the pH of blood.
To study the kinetics of this enzyme, initial rates were measured at various CO_2 concentrations in a buffer at pH 7.0. The results are shown in **Table 3**.

Using the graph paper, draw a Lineweaver-Burk (also known as a double reciprocal) plot to calculate K_m and V_{max} for the reaction.

Use the Inspera graph paper and fill in your values here.

Maximum marks: 5

3(b) A 0.1 mg/mL solution of enzyme stock was prepared for this study, and the reaction was started by adding 2 µL of this stock to the 2 mL reaction. What is the value of k_{cat}? The molecular weight of carbonic anhydrase is 30 kDa.

Fill in your answer here

Maximum marks: 2

3(c) Carbonic anhydrase uses a Zn^{2+} cofactor in its active site. The Zn^{2+} ion is coordinated by three histidine (His) sidechains and the substrate water molecule as shown in **Figure 1**, alongside a schematic of the His structure. Briefly explain how His coordinates Zn^{2+}, stating which atom in the His residue is involved.

Fill in your answer here

Maximum marks: 1

3(d) The enzyme kinetics was measured again at pH 5.0. K_m was unaffected, but k_{cat} was 10-fold lower compared to the results at pH 7.0. How would you explain this?

Fill in your answer here

Maximum marks: 2

4 Photosynthesis and the Oxygen Electrode Rationale and Tables

Section A
Rationale for Question 4 subparts a and b

You would like to test whether a herbicide affects photosynthesis. For this, you want to measure photosynthetic rate in the absence and presence of the herbicide using chloroplasts extracted from lettuce leaves. You harvest 8 grams of leaves and homogenize them in 30 mL of sucrose buffer. The homogenate is centrifuged at 3000 *g* and the pellet is resuspended in 4.5 mL of sucrose buffer.

400 µL of the chloroplast suspension are removed and mixed with 900 µL of acetone. To determine the amount of chlorophyll, you measure the absorbance of this solution at 652 nm using a spectrophotometer. You obtain an A_{652} value of 0.90.

You leave your suspension at room temperature while you have lunch with a friend. After coming back to the lab several hours later, you use your chloroplast suspension to measure photosynthetic rate with an oxygen electrode. You place 20 µL of the resuspended chloroplasts in the electrode chamber containing the electron acceptor phenyl quinone. The final volume of the electrode chamber is 1 mL.

Section B
Rationale for Question 4 subpart c

Your colleague is carrying out the same experiment with a chloroplast extract of 4.05 mg chlorophyll per gram of leaf material. They are using the electron acceptor phenyl quinone and obtain the following readings for oxygen evolution in the absence of the herbicide, shown in **Table 4**. An electrode reading of 1 mV is equivalent to 2.55×10^{-4} µmoles $O_2\,mL^{-1}$.

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

Light/Dark	Time (s)	Oxygen tension (mV)
Dark	0	202
Dark	30	208
Dark	60	212
Dark	90	215
Dark	120	220
Light	150	338
Light	180	431
Light	210	535
Light	240	640

Section C
Rationale for Question 4 subpart d and e

After adding the herbicide at the 240 s time point, your colleague has obtained the following measurements for oxygen evolution, shown in **Table 5**.

Table 5: Oxygen electrode measurements with the electron acceptor phenyl quinone in the presence of a herbicide.

Light/Dark	Time (s)	Oxygen tension (mV)
Light + Herbicide	270	738
Light + Herbicide	300	832
Light + Herbicide	330	935
Light + Herbicide	360	1041

4(a) Photosynthesis and the oxygen electrode

Please read Section A of the rationale material before completing Question 4 subparts a and b.

Multiplying the A_{652} value by 2.9 can be used to calculate the concentration of chlorophyll in $mg_{chl}\ mL^{-1}$. Calculate the amount of chlorophyll present in 1 g of lettuce leaves. Show your workings.

Fill in your answer here or use the Inspera paper.

Maximum marks: 2

4(b) When you attempt to measure oxygen evolution, you do not detect any changes in oxygen tension. Provide two possible reasons for this.

Fill in your answer here

Maximum marks: 2

4(c) Please read Section B of the rationale material before completing Question 4 subpart c.

Using the data in **Table 4**, 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.

Fill in your answer here or use the Inspera paper.

Maximum marks: 3

4(d) Please read Section C of the rationale material before completing Question 4 subpart d and e.

From the data in **Table 5**, what can you conclude about the action of the herbicide? Explain your reasoning.

Fill in your answer here or use the Inspera paper.

Maximum marks: 2

4(e) Suggest another experiment that would allow you to investigate the site of action of the herbicide further.

Fill in your answer here

Maximum marks: 1

5 *Drosophila* Genetics Data Table

Table 6: Number of flies with brown or red eyes in in males and females.

	Females		**Males**	
	Brown eyes	**Red eyes**	**Brown eyes**	**Red eyes**
F1	1450	2	5	1720
F2	795	810	780	800

5(a) *Drosophila* Genetics

The Department of Genetics *Drosophila* laboratory stocks have become contaminated with wild *Drosophila*. You discover that a single brown-eyed male *Drosophila* from the outside world has crossed to a true breeding red-eyed virgin female in a laboratory stock you were using for your Biology of Cells practical class.

Using Punnett Squares, determine the ratio of red to brown eyes you would expect to find in the F1 and F2 generations if the brown-eye phenotype were:

i) X-linked recessive,

ii) autosomal recessive.

In each case, explain your reasoning.

i) X-linked recessive: use the Inspera paper and fill our your reasoning here.

ii) autosomal recessive: use the Inspera paper and fill our your reasoning here.

Maximum marks: 5

5(b) You set up the crosses and the data that you obtain are described in **Table 6**.

What can you conclude about the inheritance of brown eyes from the analysis of the data? Use Punnett Squares to justify your answer.

Use the Inspera paper and fill out your reasoning here.

Maximum marks: 2

5(c) Explain how you would distinguish a male from a female *Drosophila* using the resources available in the Biology of Cells practical class.

Fill in your answer here

Maximum marks: 1

5(d) Why is it important to use a virgin female for the cross with the original brown-eyed male?

Fill in your answer here

Maximum marks: 1

5(e) When performing the crosses, why is it important to remove parental flies from the tubes once eggs are laid?

Fill in your answer here

Maximum marks: 1

6(a) Bacterial Plasmids

In January 2024, every person with knowledge of the Biology of Cells plasmid practical is laid low with influenza and too ill to pick up the phone. They haven't left any notes behind about the plasmids used in the practical. The only trace of the practical left behind is a mystery vial marked "plasmid mixture" and some DH5α *E. coli*, which is a plasmid-free strain.

How can the technician, using techniques available to a Part 1A BoC student, work out what phenotypes the plasmids have?

Fill in your answer here

Maximum marks: 3

6(b) Describe briefly how the technician could deduce the size of the plasmids from an agarose gel.

Fill in your answer here

Maximum marks: 2

6(c) How would the technician distinguish between colonies containing one doubly-resistant plasmid or two singly-resistant plasmids?

Fill in your answer here

Maximum marks: 1

6(d) How does the technician work out how many unique phenotypes there are and which plasmids they are associated with?

Fill in your answer here

Maximum marks: 2

6(e) Suggest two ways the technician can generate pure stocks of single plasmids.

Fill in your answer here

Maximum marks: 2

7 PCR and Exploring DNA Rationale and Figures

Section A
Rationale for Question 7 subparts a-e

You are working in the research laboratory of Subtractenbrookes Hospital. As part of an ongoing study, you have been asked you to test whether throat swabs taken from two young adults (patient 1 and patient 2) show any indications of cytomegalovirus (CMV) infection.

CMV is a DNA virus that can sometimes cause glandular fever. Neither patient is showing any outward signs of fever. However, it is not unusual to encounter asymptomatic CMV carriers. Both patients have been age and sex-matched for the study. Patient 1 is healthy and has no underlying health issues, whereas patient 2 is immune-suppressed and is currently being treated with an antiviral drug, acyclovir.

A reliable PCR-based diagnostic assay is available to detect CMV in tissue samples. In the first experiment, your reaction mix comprises CMV-specific primers A and B (below), a mixture containing 10 µM each of dATP, dCTP, dTTP and dGTP, an aliquot of lysed tissue sample from each patient, an appropriate buffer containing magnesium, and *Taq* DNA polymerase.

Primer A 5'-GTACACTGATCTGTAGATC-3'

Primer B 5'-CACAGTTAGATGACTACTG-3'

You carry out your PCR reaction (30 cycles, using an appropriate annealing temperature) and resolve the products on a 1.5% (w/v) agarose gel containing 0.6 µg/mL ethidium bromide. The results are shown in **Figure 2**.

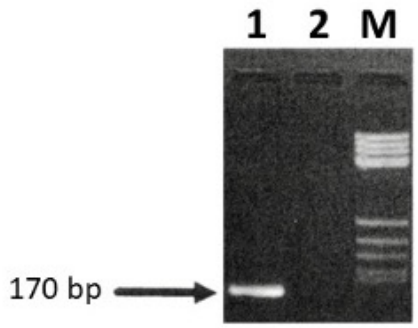


Figure 2: The PCR reaction mixtures were resolved by agarose gel electrophoresis in the presence of ethidium bromide. The resulting gel was visualized on a flat-bed UV illuminator. The expected size of the amplicon from CMV is ca. 170 bp. Lane 1; sample from patient 1, lane 2; sample from patient 2, lane M; DNA sizing ladder.

Section B
Rationale for Question 7 subparts f-g

A colleague points out that the result in **Figure 2** might indicate that the patient 2 throat swab sample contains much less input template DNA than the patient 1 sample. To test this, in a second experiment, you carry out three new PCR reactions;

- Reaction X is an exact repeat of experiment 1.
- Reaction Y is also a repeat of experiment 1, but with the input tissue sample from both patients 1000 × diluted.
- Reaction Z is also an exact repeat of experiment 1, but with both input samples spiked with purified CMV.

Following PCR amplification and resolution of the products by agarose gel electrophoresis, the results in **Figure 3** were obtained.

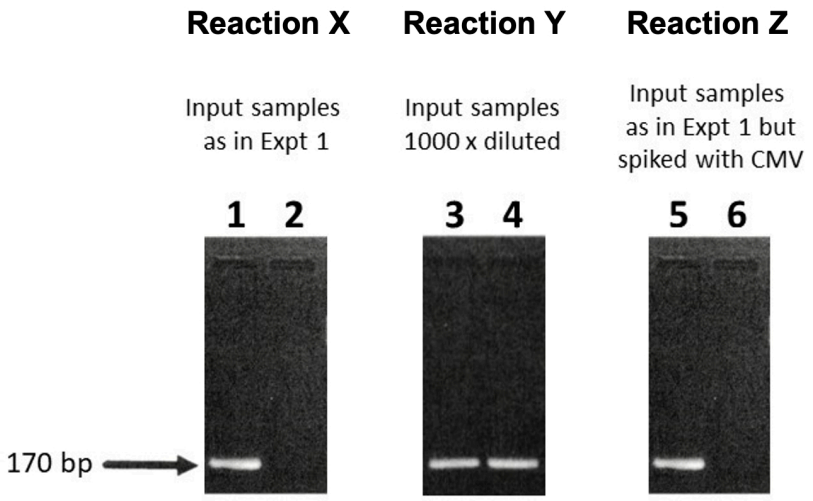


Figure 3: Products from the PCR reactions carried out in the second experiment resolved by agarose gel electrophoresis in the presence of ethidium bromide. Reaction X: undiluted tissue sample as input DNA. Reaction Y: 1000-fold diluted tissue sample as input DNA. Reaction Z: undiluted tissue sample spiked with 1 μg mL^{-1} purified CMV as input DNA. Lanes 1, 3 and 5 – tissue sample from patient 1, lanes 2, 4 and 6 – tissue sample from patient 2.

Section C

Rationale for Question 7 subparts h-j

Patient 2 is being treated with acyclovir. The structure of this widely-used antiviral purine analogue is shown in **Figure 4**, alongside two other common purine deoxynucleotides.

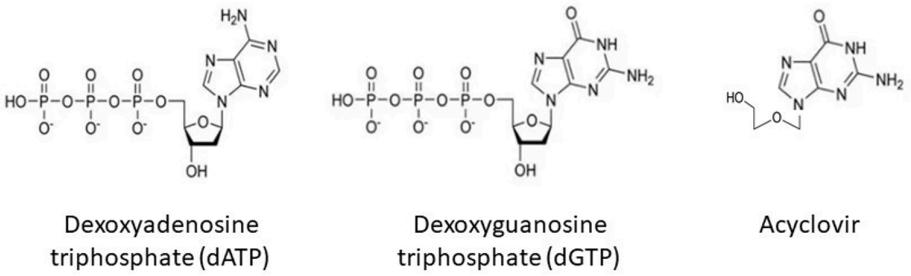


Figure 4: Structure of the antiviral agent, acyclovir, alongside two other purine nucleotides.

You decide to examine whether acyclovir affects the PCR reaction. Reaction conditions are the same as those in experiment 1 except that the template used is purified CMV. The concentrations of acyclovir tested were chosen to span the concentrations of the drug that are known to accumulate in the tissues of people taking this drug.

The results are in **Figure 5**.

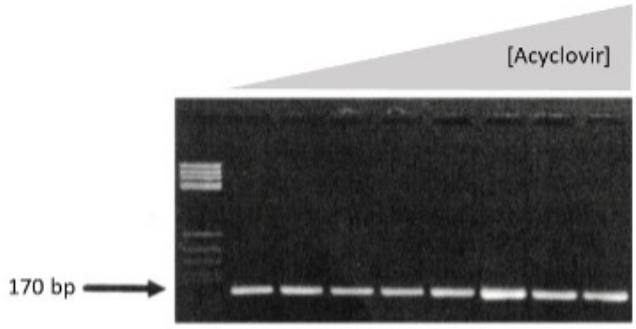


Figure 5. Effect of different acyclovir concentrations on PCR-amplification of a 170 bp fragment of CMV.

7(a) PCR and Exploring DNA

Please read Section A of the rationale material before completing sections a-e.

Assuming that every base in **primer A** is complementary with a base on the template strand, how many hydrogen bonds hold this primer to the template?

Fill in your answer here

Maximum marks: 0.5

7(b) What makes primers A and B well designed?

Fill in your answer here

Maximum marks: 3

7(c) The mean GC content of the primers is comparable (within 1%) with the mean GC content of the genome of the studied strain of CMV. What is the mean GC content of the CMV strain?

Fill in your answer here

Maximum marks: 0.5

7(d) What do you conclude from the data in **Figure 2**?

Fill in your answer here

Maximum marks: 0.5

7(e) How does ethidium bromide work?

Fill in your answer here

Maximum marks: 2

7(f) **Please read Section B of the rationale material before completing sections f-g.**

Comparing the results from reactions X and Y only, what do you conclude?

Fill in your answer here

Maximum marks: 0.5

7(g) Comparing the results from reactions X and Z only, what do you conclude?

Fill in your answer here

Maximum marks: 0.5

7(h) Please read Section C of the rationale material before completing sections h-j.

h) Based on the structures in **Figure 4**, can you suggest a possible mechanism by which acyclovir might act as an antiviral agent?

Fill in your answer here

Maximum marks: 0.5

7(i) Does acyclovir inhibit the PCR reaction?

Fill in your answer here

Maximum marks: 0.5

7(j) Thinking about your results you go down the pub with your friends. They point out that many drugs are pro-drugs that need to be converted into a bioactive form by the action of viral and cell-encoded enzymes. Do you think acyclovir might be a pro-drug? If so, how might it be modified, and by what general class of enzymes?

Fill in your answer here

Maximum marks: 1.5

8 Imaging Cell Division Figure

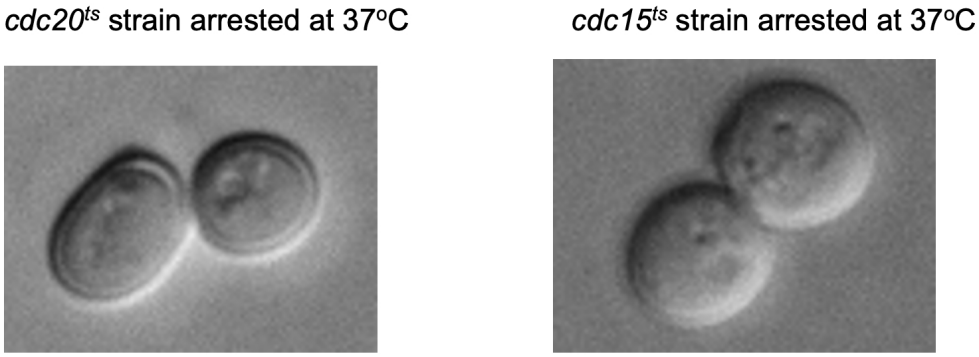


Figure 6: Differential Interference Contrast images of mutant yeast cells following a 3-hour shift to 37°C.

8(a) Imaging Cell Division

Using the immunofluorescence (IF) technique, you analyse two temperature-sensitive yeast mutants *cdc20*^{ts} and *cdc15*^{ts} arrested in mitosis at restrictive temperature.

Briefly describe the principle of the IF technique providing the main protocol steps.

Fill in your answer here

Maximum marks: 3

8(b) What are the major advantages and limitations of the IF technique relative to the use of fluorescent tags (such as GFP and derivatives)?

Fill in your answer here

Maximum marks: 3

8(c) Describe what features in the cells your antibodies need to target to distinguish the two cell cycle stages. Note: CDC20 encodes an essential activator of the anaphase-promoting complex and CDC15 encodes a protein kinase required for mitotic exit.

Fill in your answer here

Maximum marks: 2

8(d) For each strain, draw the cell shapes based on the images shown in **Figure 6** and annotate your sketch with the expected result of your experiment.

Use the Inspera paper

Maximum marks: 2

9 Embryological Zoo Figure

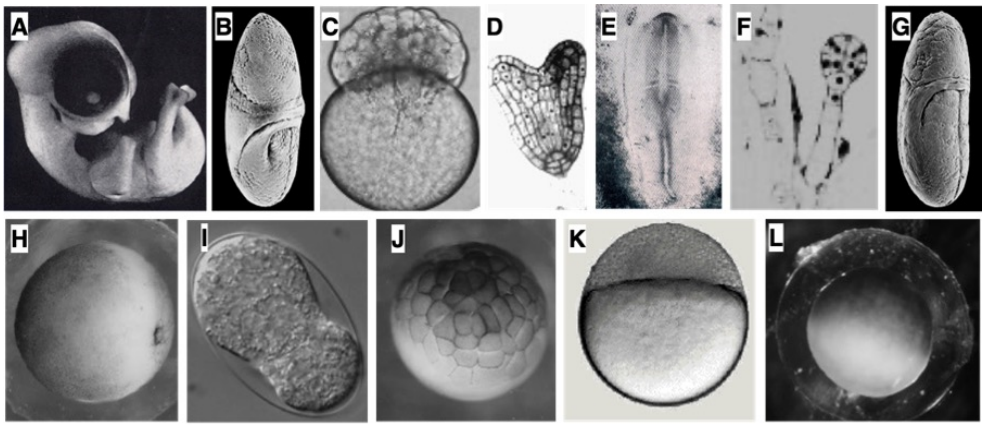


Figure 7: Stages of embryonic development in different species.

9(a) Embryological Zoo

Panels A-L of **Figure 7** show representative images of stages of embryonic development from six different organisms.

Name the organism depicted in each panel (common or Latin names acceptable).

Fill in your answer here: Panel A
Fill in your answer here: Panel B
Fill in your answer here: Panel C
Fill in your answer here: Panel D
Fill in your answer here: Panel E
Fill in your answer here: Panel F
Fill in your answer here: Panel G
Fill in your answer here: Panel H
Fill in your answer here: Panel I
Fill in your answer here: Panel J
Fill in your answer here: Panel K
Fill in your answer here: Panel L

Maximum marks: 6

9(b) Using the animal examples, which image is:

i) the comparatively earliest
ii) the comparatively latest

in development regardless of the organism?

Fill in your answer here

Maximum marks: 1

9(c) Briefly discuss why *Arabidopsis thaliana* and *Drosophila melanogaster* are more advantageous than *Danio rerio* or *Xenopus laevis* for performing a genetic screen?

Fill in your answer here

Maximum marks: 3