Faculty of Science

School of Life and Environmental Science

BCMB2001 and MEDS2003 - Biochemistry and Molecular Biology

Calculations and Theory of Practical Exam

Semester 1, 2020

Instructions

- This is due on Sunday, 3 May 2020 at 11:59 pm.
- This is a take home exam and must be completed INDIVIDUALLY.
- Questions related to this exam will NOT be answered on Piazza or email.
- Mark allocations are described on each question.

Section A: Laboratory Calculations (50 marks)
TEN short answer questions and an Excel component.
Questions 1 to 10.

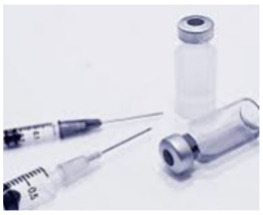
Section B: Spectrophotometry (20 marks)
FIVE short answer questions.
Questions 11 to 15.

Section C: PCR and Gel Electrophoresis (50 marks)
SIXTEEN short answer questions.
Questions 16 to 31.

Section D: Enzyme Kinetics (15 marks)
FIVE short answer questions.
Questions 32-36.

Section A: Calculations (50 marks)

All answers must be presented so that the number part is between 0.1 and 1000 and that appropriate prefixes are used to avoid the use of scientific notation



All the questions refer to an experimental drug (RDRPkill), designed to treat COVID-19. The drug is a specific inhibitor of the coronavirus specific RNA-directed RNA-polymerase.

Although it does not dissolve in pure water, RDRPkill is soluble in 10% (w/v) ethanol or pure dimethyl sulfoxide (DMSO).

It is supplied in vials that generally contain 5 mg of powder. Liquid is transferred in and out of these vials by puncturing the rubber seal at the top with a syringe.

1. (2 marks)

The pharmaceutical company media department hired a chemistry major to write some information about the drug. Edit the following statement to make their numbers more understandable.

"RDRPkill is effective in humans at 0.0003 x 10^{-3} g/kg/day, and can be stored in micro-ampules containing 8,000 nL. At plasma concentrations above 100,000 ng/L the compound is toxic. It is strongly effective at killing COVID-19 in cell culture at a concentration of 5×10^3 nmol/mg protein"

2. (5 marks)

RDRPkill has a molecular weight of 400. You have a small, concentrated 500 mM stock solution.

a. Express this concentration in the following units:
   i. M
   ii. µg/µL
   iii. % (w/v)
   iv. weight/dL
b. How much of this compound would you have to weigh out if you were to make up 500 µL of the solution?
c. If you added 5 µL of a 500 mM solution to a cell incubation with a total volume of 2 mL
   i. How many moles would the incubation contain?
   ii. What is the final concentration of this compound in the incubation?

3. (5 marks)

An experiment requires an incubation to be carried out with 1 μM of RDRPkill. The incubation will be carried out in a total volume of 500 μL of culture medium. What volume of each of the stock solutions below (a – d) would you need to add to the incubation to give 1 μM of RDRPkill?

a. 1 mM
b. 0.5% (w/v)
c. 20 μg/dL
d. 10 μg/mL

e. Due to the toxicity of the solvent, the volume added cannot exceed 0.1% (v/v) in the final 500 μL of culture. Which of the stock solutions above COULD actually be used for this incubation?

4. (3 marks)

You want to prepare a 10 mM stock solution of RDRPkill for the cell culture experiment described above. You have a 5 mg vial of this powder. You decide to make up the batch by using a syringe to puncture the rubber membrane at the top of the vial, dispensing in a solvent, dimethyl sulfoxide (DMSO). What volume of DMSO would you add to the vial?

Why would you NOT contemplate opening the vial and weighing out the powder?

5. (8 marks)

You are setting up multiple lung cell incubations. These lung cells adhere to and grow on the bottom of a plastic well as a monolayer.

This picture shows a 24-well plate. The culture medium, which comes pre-made from a commercial supplier) is added into the wells, onto the cells. Media supplements such as glucose, virus and drug are later added into the culture medium

In your experiment you will set up a 24-well plate, with 1 mL of culture medium in each of the wells. In the culture medium you will need to add glucose to a final concentration of 5 mM (to make the lung cells happy). 1,000 COVID-19 viruses will be added to 20 of the wells on each plate (this won't make the cells so happy). The incubations will take place in the absence or presence of RDRPkill (concentrations ranging from 0 to 50 μM).

The glucose is supplied as a 500 mM stock solution. The virus is supplied as a suspension which contains 1 million viruses per μL. You have your RDRPkill stock solution which is 10 mM.

a. How would you set up ONE well containing 1 mL culture medium, 5 mM glucose, 1,000 COVID-19 viruses, and 50 μM RDRPkill?
b. When setting up the entire experiment, your supervisor suggests that you make up a Master Mix containing culture medium, virus and glucose – thus meaning that you only need add about 1 mL of this to each well How would you make up that master mix?
c. Explain, in general terms and perhaps using a picture of the plate or a table, how you would organise the plate.

6. (4 marks)

Because everyone in the world is using lung cell culture medium, there is a shortage. But, no problem, you can make a homemade version using basic components. The *final concentration* of the components in culture medium are 130 mM sodium chloride, 2.5 mM potassium chloride, 25 mM sodium bicarbonate, and 100 µg/mL BSA.

You need to prepare a *10 x concentrate of culture medium*. You have the following stock solutions: 5 M sodium chloride, 0.5 M potassium chloride, 2.5 M sodium bicarbonate, and 30% (w/v) BSA. How would you make up 200 mL of this *10 x concentrate*?

7. (3 marks)

It turns out that RDRP-kill may need to be stabilised in the incubations by a reducing agent. A commonly used reducing agent is β-mercaptoethanol (mol.wt. = 78.3). It is a liquid at room temperature with a density of 1.12 g/mL. You decided that you will make up a 50 mL stock of 0.7% (v/v) β-mercaptoethanol. How would you do this and what is the molar concentration of the stock that you've made up?

8. (2 marks)

For some types of downstream an analysis, you will terminate the lung cell incubations by adding a strong acid. The acid is also great for disposal of culture medium that contains virus. This acid is supplied as a liquid with a density of 1.4 g/mL and a purity of 75%. If the molarity of the solution is 15 M what is the molecular weight of the acid?

9. (6 marks)

At the end of most of the lung cell incubations, you will isolate RNA. To do that you will take off all the medium (being careful to dispose of it carefully!!!). Then, after rinsing the wells with saline, you'll add a mixture of chemicals which smash open the cells and release the RNA.

The solutions that do this contain chaotropic compounds like guanidinium salts which denature macromolecules by disrupting H-bonding. The RNA isolation solution also contains a reducing agent (the aforementioned β-mercaptoethanol) to denature disulfide bonds.

As with the culture medium, the commercial 'kit' 'ready-made' cocktail of chaotropic agents (called Tri-reagent) is in short supply. So we'll make our own alternative.

The final composition of the solution is: 4 M guanidine isothiocyanate (GITC), 25 mM sodium citrate, pH 7.0, 0.5 % (w/v) SDS, 0.1 M β-mercaptoethanol.

You have guanidine isothiocyanate powder (mol. wt. 118.16), 0.5 M sodium citrate, pH 7.0, 10% (w/v) SDS (mol. wt. 288.4) and β-mercaptoethanol (density 1.12 g/mL, mol. wt. 78.1).

How would you make up 200 mL of this solution?

10. (12 marks total)

Miraculously a colleague is a hoarder of Tri-reagent and is willing to let you have some in exchange for some toilet rolls, tins of tomatoes and tubes of hand sanitizer (only joking about the latter, you just steal a bottle of neat ethanol from the Biochem store). This means you can now make your RNA according to the standard protocol as shown below.

RNA Extraction Protocol

1. Remove media (biosafety cabinet, dispose in flask full of strong acid)
2. Wash with 1 mL saline (again, dispose of washings properly)
3. Add 500 µL Tri-reagent to wells (fume hood). Shake plate gently. Check that cells are mobilised (microscope).
4. Pipette up and down (~20 times) until the mixture becomes less viscous. Transfer all liquid to labelled microfuge tubes.
5. Add 100 µL chloroform (ratio is 200 µL chloroform / 1 mL Tri-reagent).
6. Vortex until mixed (30 seconds).
7. Centrifuge for 30 minutes at 4°C (cold room) at max speed (14,800 rpm)
8. Remove the upper RNA-containing layer (~150-250 µL) and transfer to a new microfuge tube.
9. Add 500 µL isopropanol (same amount as Tri-reagent). Invert to mix.
10. Store at -20°C overnight (or a few hours at -80°C).
11. Spin for 20 minutes at 4°C at 14,800 rpm. Discard supernatant.
12. Add 500 µL 80% ethanol, vortex briefly (can store overnight at -80°C) centrifuge for 20 minutes at 4°C at 14,800 rpm. Discard supernatant. Repeat.
13. Air dry pellet for 5-10 minutes (if still wet, do a quick spin, then pipette off the liquid).
14. Re-suspend pellet in 12 µL TE (Tris-EDTA buffer).
15. Measure RNA purity and yield with Nanodrop**.**

    Ideally the Nanodrop spectrum will show a peak at A260

    - A260/A280 < 1.8 (less means probable protein contamination)
    - A260/A230 < 1.8 (less means probable reagent/salt contamination
    - A peak at A270 means phenol contamination

Assuming that the RNA samples are of sufficient yield and purity, the next step is to make cDNA from the RNA. This requires 500 ng of the RNA, where the maximum volume of RNA we can add to a 20 µL reaction is 12 µL.

a. Draw a picture of the process, clearly annotating where the RNA is at each step. (4 marks)

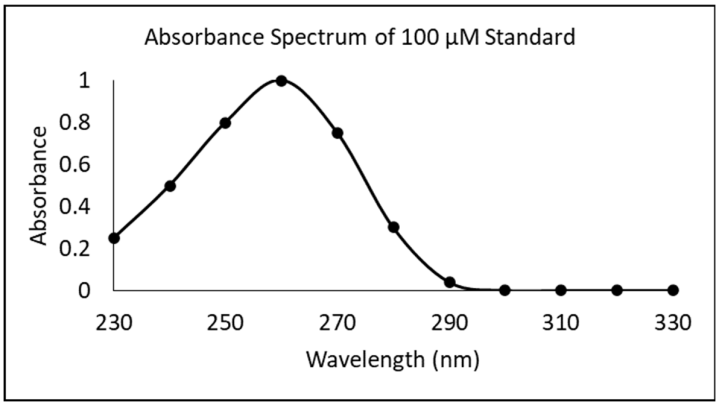
b. Using the data below, create an Excel spreadsheet that (8 marks)
   i. calculates the yield (amount) of RNA from each well (sample)
   ii. calculates the volume of each RNA preparation required for the cDNA synthesis
   iii. flags the samples which should not (or cannot) be used for the cDNA synthesis (HINT: investigate how to use the IF function or conditional formatting)

Sample ID	ng/µL	A260/A280	A260/A230
A	610.75	2.03	1.65
B	5.88	2.06	2.07
C	613.69	2.05	1.76
D	574.69	2.07	1.56
E	456.49	2.01	2.02
F	567.07	2.06	1.57
G	543.25	1.99	1.43
H	3.76	2.04	2.1
I	601.97	2.06	1.78
J	0.73	2.03	1.48
K	557.28	2	0.76
L	474.55	1.98	2.13
M	573.01	2.08	0.98
N	508.82	1.99	1.13
O	611.49	2.01	1.64
P	448.46	2.01	1.95
Q	38.61	1.73	0.11
R	20.98	1.5	0.46
S	31.14	1.46	0.68
T	51.55	1.77	0.92

Section B: Spectrophotometry (20 marks)

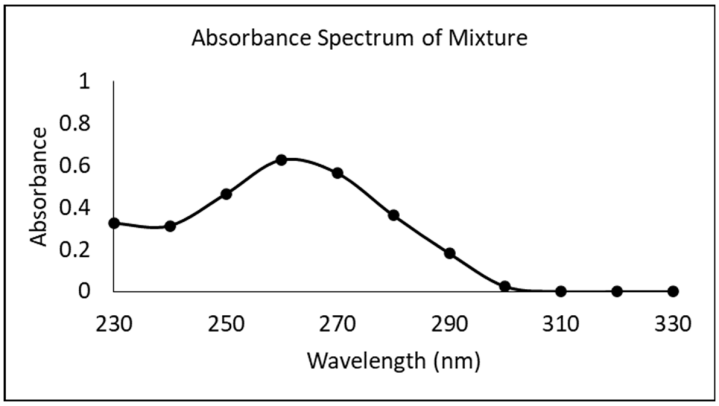
All responses in this section must include an accompanying explanation.

You are trying to quantify the amount of an adenosine analogue in a tablet to be used as an antiviral drug. This was solubilised to produce a colourless solution that absorbs strongly at 260 nm. You also have a 100 µM standard of this compound, with the following absorbance spectrum:



Nothing else in the tablet (i.e., the filler) absorbs within this range (between 230 nm and 330 nm).

11. You have taken 1 mL of this standard in a test tube, with the intention of validating that the standard curve is as described. However, your lab partner added 1 mL of a colourless solution to this while your back was turned. They have no idea what they added, but the mixture produced the following absorbance spectrum:



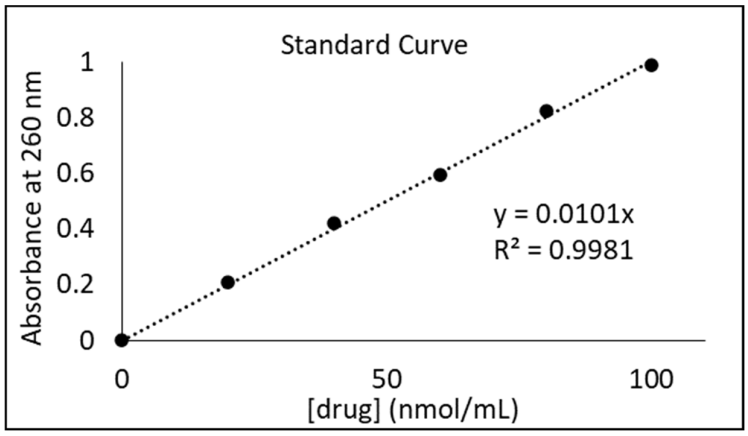
a. How have the concentration and number of moles of the antiviral drug in this mixture changed from the original 1 mL standard? (2 marks)

b. What possible solutions could your lab partner have added to the test tube? (3 marks)

12. You have performed dilutions of the 100 µM standard. Your lab partner has set the spectrophotometer to photometric mode, transferred the 100 µM standard into a quartz cuvette, and pressed start. However:
    a. They are getting zeroes for their absorbance.
    b. They are getting 2.4 for their absorbance.

What might be the reasons for this? (2 marks each)

13. You plotted the following standard curve using dilutions of your standard:



Separately, your lab partner has calculated the extinction coefficient of 0.012 $mM^{-1}cm^{-1}$.

Another peer is saying that the extinction coefficient is 10 $mM^{-1}cm^{-1}$.

How have these two students arrived at these values? Who is correct? (3 marks)

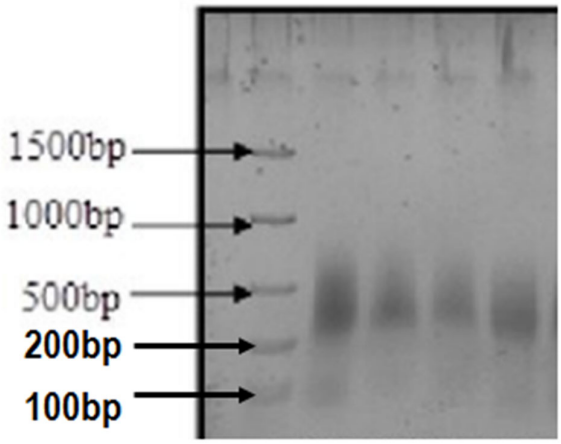
14. You want to calculate the concentration of the dissolved tablet. You already know that the concentration will range from 1-2 mM.
    a. Describe a strategy to achieve some suitable dilutions. (2 marks)
    b. Your lab partner suggests that you can just add the same volume, three times and use these triplicates to calculate the concentration. How do you respond to this idea? (3 marks)

15. You want to measure how quickly this drug is cleared from the system after it has been administered, and are considering using spectrophotometry. What are some considerations when working with serum samples? (3 marks)

Section C: PCR and Gel Electrophoresis (50 marks)

As a student, you have been asked to design an experiment to determine the size of a variable number tandem repeat region within the human genome. The D1SGARETH region is known to have between 3 and 21 identical tandem repeat sequences (16bp each) flanked by conserved sequences (115bp upstream and 32bp downstream of the tandem repeats).

You isolate the DNA from the cheek cells of 4 students in your laboratory and perform a set of PCRs to amplify the region of interest using the extracted DNA as a template. Agarose gel electrophoresis was performed to separate the amplified products. The gel was stained with HydraGreen and photographed.



Lane (numbered left to right)	Sample loaded
1	DNA Ladder
2	PCR - student 1 DNA template
3	PCR - student 2 DNA template
4	PCR - student 3 DNA template
5	PCR - student 4 DNA template

Figure: Agarose gel electrophoresis of PCR amplicons from 4 individuals. Lane 1 (left) was a DNA ladder of known fragment sizes (bp).

Electrophoresis Troubleshooting (5 marks)

This was the very first time that you have performed an agarose gel electrophoresis on your own and the results are not what you had expected.

Using the buffers in the laboratory, you weighed out 1.0 g of agarose powder and made it up to 100 mL with TBE (Tris-Borate-EDTA) buffer.

You heated this to dissolve the agarose and poured it carefully into the gel tank. Once the agarose had set (approximately 30 mins), you added just enough of the same TBE buffer to the tank to fully submerge the gel and removed the comb, exposing the wells in which your samples were loaded.

Samples were combined with 'stop mix' loading dye, and these were carefully loaded into each of the 5 wells.

You set the power pack to 80 volts for 1 hour to separate the DNA samples. After disconnecting the power pack, the gel was stained with HydraGreen for 20 minutes, and photographed using a UV transilluminator.

You think that you have followed the instructions carefully, but the bands on your gel don't look very clear. You ask the PhD student in the laboratory for some advice and they respond with a number of questions:

"There's something wrong with the bands in my agarose gel. Could you please help me?"

16. "Did the hydra green stain work? Did it bind to the DNA? Did it have time to intercalate? How do you know?" (1 mark)

17. "What does the ladder tell you about the gel electrophoresis?" (1 mark)

18. "Were you able to estimate the size of the PCR amplicons for each student?" Y/N? Why? (2 marks)

19. "So now you have collected lots of information. Did your agarose gel run correctly?" (1 mark)

PCR Troubleshooting (20 marks)

The PhD student was helpful. You think that there were no major problems with the gel. So, you begin to ask about the PCR, but the PhD student says...

"I am hopeless with PCR, I can never get it to work properly. Ask the Postdoc Alice, she is a genius with everything related to PCR!"

So, you approach the Postdoc with your gel photo and your PCR protocol.

"I am having trouble with my PCR. Can you please help me?"

"Sure, what's the problem?"

"I ran the PCRs and checked my results on an agarose gel, but the bands are not what I had expected"

Alice tells you that you definitely need positive and negative controls for your PCR.

Outline below what you might have included as positive and negative controls. What would these have told you about your unexpected results?

20. What could you use as positive control(s)? (2 marks)

21. What could the positive control(s) tell you about your experiment? (3 marks)

22. What could you use as negative control(s)? (2 marks)

23. What could the negative control(s) tell you about your experiment? (3 marks)

"Are you sure that you added every component to your PCRs? Some of the PCR reagents in the lab have been in that freezer for many years. How do you know that they are still working?"

24. List the ingredients that you would have added to each of the student PCRs. Next to each component, indicate if there is any evidence: (a) that you remembered to add this to your reaction and (b) that it was not denatured, degraded or contaminated in a manner that would have compromised the success of the PCR (10 marks)

PCR component	**Working Y/N?**	**Evidence**
e.g. Taq polymerase	Y	There appears to be PCR products on the gel

Primer Design Troubleshooting (25 marks)

The postdoc was very helpful. You think that there were no major problems with the PCR. So, you start to look back at the sequence that you were trying to amplify. You ask the Postdoc for help but she says "My supervisor always works out all of those details – I'm not sure how to design the PCR. I just set up the reactions and run the gels. Ask Dale, she knows everything about PCR design!"

So, you approach Dale with all of the evidence that you have gathered so far.

"I am having trouble with my PCR. I am CONFUSED! Can you help me?"

"Why don't you post your question to Piazza?"

"But I need your help now and the question relates specifically to my set of results"

“Very well then, what is the problem?”

“I am trying to amplify the D1SGARETH region from some DNA that the students have extracted from their cheek cells. I looked up the human genome sequence on the internet, and this region has between 3 and 21 repeats of the same 16bp sequence (GAAGACCACCGGCAAG).

On either side of these repeats, there are conserved sequences that are the same in everyone’s DNA. On the coding strand there are 115 bp of conserved sequence at the 5’ end and 32 bp of conserved sequence at the 3’ end.

Repeat region “D1SGARETH” is shown below

5’ AAACTGGCCT CCAAACACTG CCCGCCGTCC ACGGCCGGCC GGTCCTGCGT GTGAATGACT
CAGGAGCGTA TTCCCCACGC GCCAGCACTG CATTCAGATA AGCGCTGGCT CAGTG|

GAAGACCACCGGCAAG| GAAGACCACCGGCAAG| GAAGACCACCGGCAAG|
GAAGACCACCGGCAAG| GAAGACCACCGGCAAG| GAAGACCACCGGCAAG|
GAAGACCACCGGCAAG| GAAGACCACCGGCAAG| GAAGACCACCGGCAAG|
GAAGACCACCGGCAAG| GAAGACCACCGGCAAG| GAAGACCACCGGCAAG|
GAAGACCACCGGCAAG| GAAGACCACCGGCAAG| GAAGACCACCGGCAAG|
GAAGACCACCGGCAAG| GAAGACCACCGGCAAG| GAAGACCACCGGCAAG|
GAAGACCACCGGCAAG| GAAGACCACCGGCAAG| GAAGACCACCGGCAAG|

CC TGCAAGGGGC ACGTGCATCT CCAACAAGAC 3′

Figure: Human D1SGARETH VNTR amplicon: - with 21 repeat units shown in **BOLD** (known alleles contain between 3 and 21 repeat sequences). The repeat sequences are separated by “|”. The highlighted regions are the primer binding sites. Notice that there are regions before and after the repeats, called the flanking regions. These are the same in every allele.

I want to know how many repeats each of these students have in their genomic DNA.

If I can tell the students apart based on their DNA samples, we will no longer need to worry about webcams and technology for proctored exams. We can simply swab their keyboards and collect some skin cells to confirm their identity directly from their DNA – simple!”

“To fix your PCR problems I need to know the sequence of the two primers you are using and I need to know the sequence that you are trying to amplify. Bring these to me now. Show me exactly where your primers are binding to the sequence that you are trying to amplify.”

25. Below are the sequences of the primers that you have ordered for your experiment. In the double-stranded template sequences below, show Dale exactly where your primers are going to bind during the annealing step of the PCRs (indicate every possible binding site for each of your primers). (5 marks)

> primer 1 sequence consisting of 27 nucleotides (single-stranded)

5’ AAACTGGCCTCCAAACACTGCCCGCCG 3’

> primer 2 sequence consisting of 16 nucleotides (single-stranded)

5’ CTTGCCGGTGGTCTTC 3’

> conserved 5’ flanking DNA sequence consisting of 115 bp

5’ AAACTGGCCTCCAAACACTGCCCGCCGTCCACGGCCGGCCGGTCCTGCGTGTGAATGACTCAGG
3’ TTTGACCGGAGGTTTGTGACGGGCGGCAGGTGCCGGCCGGCCAGGACGCACACTTACTGAGTCC

AGCGTATTCCCCACGCGCCAGCACTGCATTCAGATAAGCGCTGGCTCAGTG 3’
TCGCATAAGGGGTGCGCGGTCGTGACGTAAGTCTATTCGCGACCGAGTCAC 5’

> 3 (minimum number) Variable Repeats consisting of 48 bp

5’ GAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAG 3’
3’ CTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTC 5’

> 21 (maximum number) Variable Repeats consisting of 336 bp

5’ GAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAG
3’ CTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTC

GAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAG
CTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTC

GAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAG
CTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTC

GAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAG
CTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTC

GAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAGGAAGACCACCGGCAAG
CTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTCCTTCTGGTGGCCGTTC

GAAGACCACCGGCAAG 3’
CTTCTGGTGGCCGTTC 5’

> conserved 3’ flanking DNA sequence consisting of 32 bp

5’ CCTGCAAGGGGCACGTGCATCTCCAACAAGAC 3’
3’ GGACGTTCCCCGTGCACGTAGAGGTTGTTCTG 5’

You return to Dale with your answers. And just as you expected, she has more questions for you...

“What exact size PCR product(s) did you expect to see after the gel electrophoresis?”

“I’m really not sure, because I don’t know how many repeats would have been in the student samples!”

26. “Alright, let’s simplify my question. If the student were homozygous, and had the minimum number of 3 repeats of the GAAGACCACCGGCAAG sequence in their genomic DNA, what size PCR product(s) would you expect to see?” (2 marks)

27. “Now, let’s consider if the student were homozygous, and had the maximum number of 21 repeats of the GAAGACCACCGGCAAG sequence in their genomic DNA, what size PCR product(s) would you expect to see?” (2 marks)

28. “For a heterozygous individual with 3 and 21 repeats of the GAAGACCACCGGCAAG sequence in their genomic DNA, how many different PCR products would you expect to generate in the reaction that you set up?” (2 marks)

29. “Do your answers to these questions explain your observations in the DNA gel electrophoresis?” Now that you have completely analysed your PCR, use this information to explain the unexpected bands observed in your agarose gel electrophoresis. (4 marks)

30. “What changes might you make to your experimental design based on all of this troubleshooting?” (6 marks)

31. “Would this be enough information to ascertain the identity of the student sitting their final exam by sampling their keyboard for DNA from skin cells? Explain your reasoning.” (4 marks)

Section D: Enzyme Kinetics (15 marks)

You are investigating the kinetics of Arctic shrimp Alkaline Phosphatase. As part of its evolutionary adaption to extremely cold environments the shrimp has an alkaline phosphatase which shows high activity at very low temperatures (0 to 10°C). You plan to explore the kinetics of this shrimp enzyme and compare it to the standard alkaline phosphatase.

You have a phosphorylated substrate X-P, which produces a red product, X, under the action of alkaline phosphatase. The extinction coefficient for this red product is 4 $mM^{-1}cm^{-1}$ at 500 nm.

You set up a 1 mL assay in 0.1 M glycine buffer, pH 9.5, containing 5 mM $MgCl_2$ and 0.5 mM substrate X-P. You blank the spectrophotometer and add 20 µL of an enzyme preparation containing 0.1 µg of shrimp alkaline phosphatase. After 5 min, the absorbance at 500 nm reached 0.6.

Once you have the enzyme assay working reliably you measure the rate of the reaction (velocity) at a full range of substrate concentrations (0.1 – 500 mM). The results are shown in the table below:

[Substrate X-P] (mM)	**Velocity (nmol/min)**
0	0
0.1	20.0
0.2	30.0
0.5	42.8
1.0	50.0
2.0	54.6
5.0	57.7
10.0	58.8
50.0	59.8
250.0	60.0
500.0	60.0

Using this stimulus material, and your understanding of enzyme kinetics, create one TRUE and one FALSE statement in reference to the five prompts:

32. What happens if the time course is run for an extended period of time

33. How the rate of the reaction is calculated

34. How much substrate is left in the assay at a particular time

35. How the K_M and V_{max} can be estimated without formally plotting the data

36. Factors that might affect the measured K_M and V_{max}

The two statements will be worth 1 mark each, with an additional mark awarded for the suitability of the FALSE statement as a distractor in a multiple choice question. The false statements should be plausible and ideally target common misconceptions related to the topic. All statements should be brief, as would be befitting of an MCQ option