Singapore Biology League 2024

Main Problems and Answers

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Problems

P01: Belobog Camping Trip

(160 points)

Welcome to Belobog, the last bastion of the planet Jarilo-VI. 700 years ago, a phenomenon known as the Eternal Freeze enveloped the entire planet in blistering ice. The once-green and bountiful ecology was buried in a snowy grave, and most of the human population with it. Only the city of Belobog survived the disaster, but…

"...but despite having to weather the harsh climate, humanity persevered and lived to see the sunrise again!" She smiles as she hands you a tuna sandwich (Figure 1). *"Well, all that's history now. After you and your gang managed to conquer the Eternal Freeze, the global climate is slowly but surely returning back to normal!"*

Figure 1: A tuna sandwich.

That's right. You and your gang managed to conquer the Eternal Freeze with your determination, perseverence, and plot armour. You grin in pride as you bite into the tuna sandwich (Figure 1).

Oh yeah, that's Lynx. She's a camping and ecology enthusiast that you had met while you were journeying through Belobog. The two of you are on a camping trip in the middle of nowhere to study the soon-to-be-blossoming ecology.

"So, ready to head out?" She reaches out for your hand to pull you up from your comfortable camping chair. You remind yourself that this is not a date. *"Let's go~"*

Day 1: Animal spotting

Dear diary, I am actually bored to death and I am only here to spend time with Ly-

"Hey!" Lynx interrupts your inner monologue as she throws you a pair of binoculars. *"Look at that!"*

Q1. Through the binoculars, you spot several interactions between living things. Match each observation with the name of the corresponding biological interaction (A-G) between the underlined species. **(40 points)**
(Match the correct letter to the correct row.)

A. Predation
B. Mutualism
C. Commensalism
D. Parasitism
E. Amensalism
F. Competition
G. Neutralism

Observation	**Interaction (A-G)**
A lion and a hyena are practically wrestling for a dead horse on the ground. Is there a point in beating a dead horse?	
A bee collects nectar from a flower. How sweet! Well, literally.	
A cow walks through the field, crushing the poor, poor grasshoppers that live in the grass. Bummer.	
The same cow grazes the field, exposing the very dead grasshoppers to the birds holding a flyer that reads "*Grasshopper Buffet (Courtesy of Cow.)*"	

"Wow! Staring at these animals and how they interact… ah~ I love ecology." Lynx says unironically. *"Isn't this so fun?"*

You cannot help but nod as Lynx passes you a tuna sandwich (Figure 1).

Day 2: More animal spotting

Dear diary, turns out I REALLY hate camping but Lynx is honestly so nice to m-

"Oh, wow! What a cute rabb-" Lynx interrupts your train of thoughts, before she herself was interrupted by an unfortunate incident involving a fox. "*Never mind, what a mutilated rabbit."*

Suddenly, Lynx grabs your hand and pulls you towards a bush. *"Shh! Here, take these binoculars again."*

Both of you stare at animals for hours before Lynx draws up a food web based on your observations. How romantic.

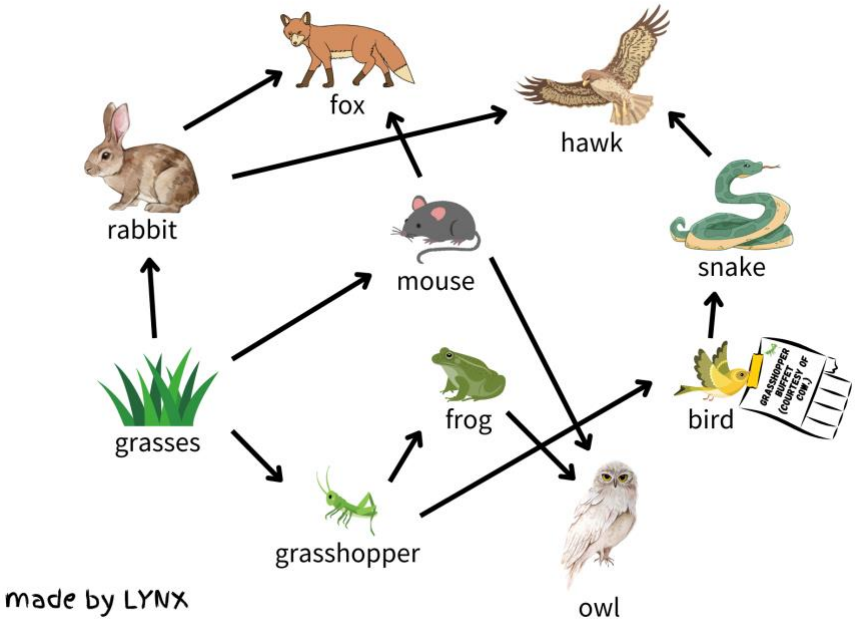


Figure 2: A food web designed by Lynx.

Q2. With reference to Figure 2, select all the secondary consumers from the list below. **(50 points)** *(Select all correct options.)*

A. Fox
B. Hawk
C. Rabbit
D. Mouse
E. Snake
F. Grasses
G. Frog
H. Bird
I. Grasshopper
J. Owl

You are sad because you have watched many animals die in the course of several hours. You have developed a newfound resolve to solve such ecological injustice! You take out your handheld Death Note and prepare to write down the names of the apex predators. They must pay for their wrongdoings!

> **Q3**. Using the first letter of the common names of the species as shown in Figure 2 (e.g. "C" for cat), order the apex predators by their trophic levels from lowest to highest. For example, if you think the answer is "Cat (lowest), Dog, Elephant (highest)", type "CDE". **(30 points)**
> *(Enter a string of letters.)*
>
> **Q4**. State the number of trophic levels in the longest food chain. **(10 points)**
> *(Enter a whole number.)*

Turns out your handheld Death Note was a dud. The guy at the marketplace must have been a swindler! With a sigh of disappointment and resignation of your previously newfound resolve, you reach into Lynx's pocket and grab a tuna sandwich (Figure 1).

Day 3: More animal spottin— ah crud, we're lost in a forest

Dear diary, we have been walking for two months. We are out of water, food and lov-

"Huh." Lynx suddenly says. *"We are no longer in a snowy biome. Where on earth are we?"*

Suddenly, someone appears from the shadow. He looks like a cross between a human and a fennet. The human fennet suddenly opens his mouth. *"Oh, hey there travellers! I'm Tighnari, a researcher in the Avidya Forest which you are standing on right now!"*

Avidya Forest?! You are so surprised at the fact for some reason that you retreat to the top of a tree.

"Huh. We must have been walking for so long we crossed into a whole different game- ah wait, breaking the fourth wall isn't allowed here!" Lynx says as she stares into a camera.

You have retreated to the top of a tree. It is safe, cozy, comfy, and absolutely perfect for some rest. Wait, no. You don't know how to get down. You scream for help.

"Ah, don't worry. Here, have some diagrams I drew just now. Maybe do some science while I come save you." Lynx says as she throws a crumpled piece of paper (Figure 3) at you.

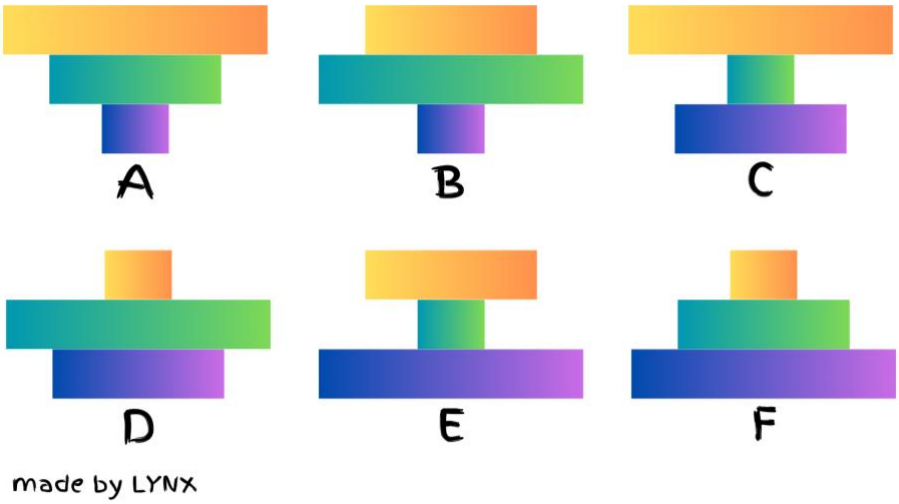


Figure 3: Several pyramids (labelled A to F) that Lynx drew on paper.

Q5. You are stuck on top of a tree. Lynx is climbing the tree to try and save you. Meanwhile, you stare at a ladybug happily munching on a leaf from the tree. It fills you with determination. Unfortunately, a bird holding a flyer that reads "*Grasshopper Buffet (Courtesy of Cow.)*" is illiterate and eats the ladybug instead. For this particular food chain in the entire Avidya Forest, state the pyramid in Figure 3 (A-F) that most likely matches the pyramids of numbers, biomass, and energy respectively. **(30 points)**
(Enter the correct letter to the correct row.)

tree → ladybug → bird	
Pyramid	**A-F**
Pyramid of numbers	
Pyramid of biomass	
Pyramid of energy	

Hurray. You are saved by Lynx. You thank her profusely.

"Oh, don't worry about it! Shall we call it a day?" Lynx says while you both sit on a tree branch. *"Well, I'm a little hungry. How about a snack?"*

Lynx pulls out a tuna sandwich (Figure 1) for herself and a tuna sandwich (Figure 1) for you. You finish the tuna sandwich (Figure 1) in two bites. Then, the two of you decide to call it a day and take a Teleport Waypoint back to Belobog City. What an adventure!

Figure 4: Alternative photo of a tuna sandwich that cannot be referenced as this figure is all the way at the bottom of the problem.

Answers and Explanations

Q1.

Answer: **F, B, E, C**

Explanation:

A. This interaction affects both the lion and hyena negatively because the presence of the other reduces the amount of food each can consume.
B. The bee collects nectar from the flower for food, and in the process, it pollinates the flower, which helps the plant reproduce. This interaction benefits both species: the bee gains nourishment while the flower receives assistance with pollination, which is essential for its reproductive success.
C. The grasshoppers are crushed and killed by the cow's movements, which negatively impacts the grasshoppers. However, the cow remains unaffected by this interaction, as it neither gains nor loses anything from the presence of the grasshoppers.
D. As the cow grazes, it disturbs the grass and exposes dead grasshoppers, which the birds then eat. The birds benefit by having an easy meal, while the cow remains unaffected by the presence of the birds or the consumption of the grasshoppers.

Q2.

Answer: **A, B, G, H, J**

Explanation: Secondary consumers are species that feed on primary consumers. Primary consumers are species that feed on primary producers. From the diagram, we find the following information:

<u>Primary producers</u>

- Grasses

<u>Primary consumers</u>

- Rabbit (feeds on grasses)
- Mouse (feeds on grasses)
- Grasshopper (feeds on grasses)

Secondary consumers

- Fox (feeds on rabbit)
- Hawk (feeds on rabbit)
- Frog (feeds on grasshopper)
- Bird (feeds on grasshopper)
- Owl (feeds on mouse)

Q3.

Answer: **FOH**

Explanation: Apex predators are species that do not have any natural predators. In this case, the fox, hawk, and owl are apex predators. The fox has a trophic level of 3 *(grasses, rabbit, fox)*, the hawk has a trophic level of 5 *(grasses, grasshopper, bird, snake, hawk)*, and the owl has a trophic level of 4 *(grasses, grasshopper, frog, owl)*.

Q4.

Answer: **5**

Explanation: The longest food chain has a trophic level of 5: Grasses, Grasshopper, Bird, Snake, Hawk.

Q5.

Answer: **B, F, F**

Explanation: Pyramids are read from the bottom being the lowest trophic level to the top being the highest.

Pyramid of Numbers

In this food chain (tree → ladybug → bird), the base represents the trees (producers), which are fewest in number because each tree can support many ladybugs and birds. The ladybugs (primary consumers) are more numerous than trees as they rely on trees for food and habitat. The number of birds (secondary consumers) is fewer than the number of ladybugs because each bird requires multiple ladybugs to sustain itself, resulting in fewer individuals at higher trophic levels.

Pyramid of Biomass

Trees a have large and significant mass. Ladybugs, despite being more numerous, have a much smaller individual mass, resulting in a narrower middle section. Birds have even less biomass than ladybugs because there are fewer of them, and they are smaller overall compared to the combined mass of the ladybugs they consume. This shows a decrease in biomass from the bottom to the top due to the lower total mass of organisms at higher trophic levels.

Pyramid of Energy

Energy decreases significantly at each successive level because a large portion is lost as heat and through metabolic processes. Only a fraction of the energy stored in trees is transferred to the ladybugs that consume parts of the trees. Even less energy is transferred to birds because they receive energy indirectly through eating ladybugs.

Credits

Figure 1: Bauer, E. (2024, January 16). *This secret ingredient makes for the best ever tuna salad sandwiches*. Simply Recipes. https://www.simplyrecipes.com/recipes/tuna_salad_sandwich/

Figure 4: Gritzer, D. (2024, March 13). *How to upgrade the classic Mayo-packed Tuna Salad Sandwich*. Serious Eats. https://www.seriouseats.com/classic-tuna-salad-sandwich-recipe

P02: Ramachandran Plots

(110 points)

In their 1963 paper titled "*Stereochemistry of polypeptide chain configurations*", G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan developed the Ramachandran plot. A Ramachandran plot is a way to visualise energetically-allowed regions for backbone dihedral angles Ψ (psi) against ϕ (phi) of amino acid residues in a protein structure. This is because atoms of the polypeptide can rotate about the N-C bond (Ψ angle) and the C-C bond (ϕ angle).

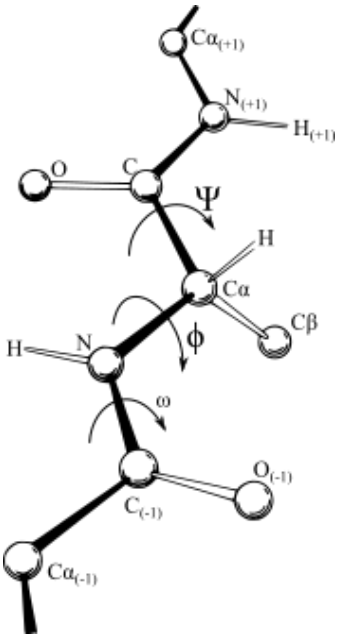


Figure 1: Backbone dihedral angles Ψ (psi) against ϕ (phi)

Figure 2 shows three Ramachandran plots. The blue area represents favourable dihedral angles while the green area represents allowed, but unfavourable dihedral angles. A larger shaded area indicates that the amino acid has a wider range and greater number of combinations of dihedral angles allowed, due to less steric repulsion between the neighbouring atoms.

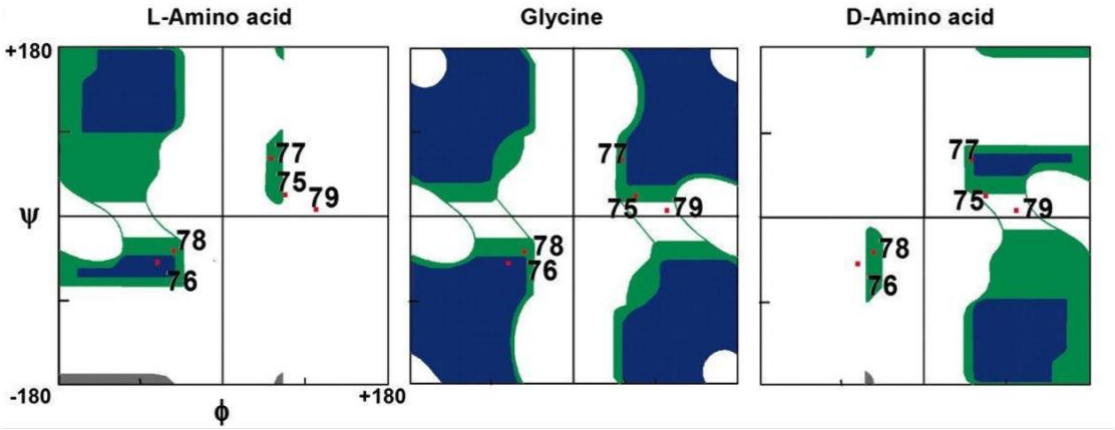


Figure 2: Ramachandran plot

Q1. By observing Figure 2 and considering the structure of glycine, indicate which statement most accurately describes the resemblance of the Ramachandran plot of glycine to that of a random L-amino acid and a random D-amino acid. **(10 points)**
(Select the correct option.)

A. The Ramachandran plot of glycine resembles that of L-amino acid more than that of D-amino acid.
B. The Ramachandran plot of glycine resembles that of D-amino acid more than that of L-amino acid.
C. The Ramachandran plot of glycine resembles that of L-amino acid as much as that of D-amino acid.
D. Glycine does not have a Ramachandran plot.
E. Glycine has an infinite number of possible Ramachandran plots.

Q2. By considering their structures, indicate which one of the following amino acids will likely have the greatest coloured region on the Ramachandran plot. **(10 points)**
(Select the correct option.)

A. Arginine
B. Alanine
C. Asparagine
D. Cysteine
E. Glutamic acid
F. Glycine
G. Histidine
H. Isoleucine
I. Leucine
J. Tyrosine

Since amino acids also contain a strongly acidic carboxyl group as well as an amino group, they can be titrated against an alkali. The titration curves of all 20 canonical amino acids are seen in Figure 3.

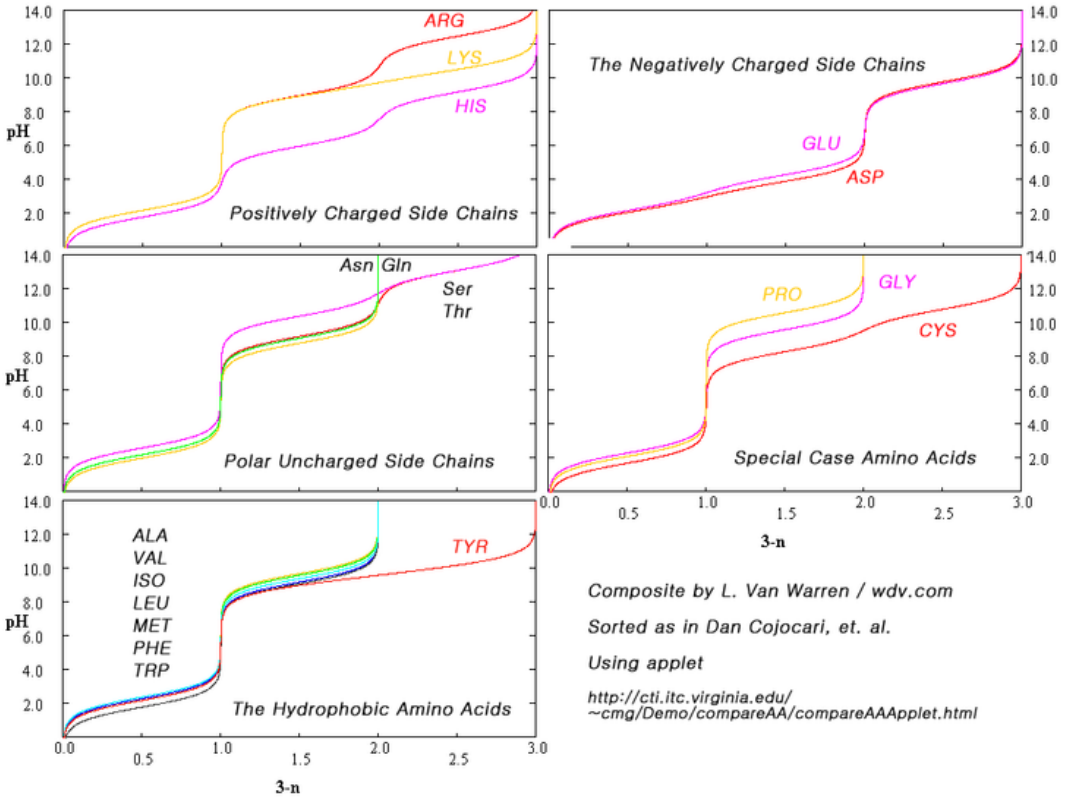


Figure 3: Titration Curves of the 20 canonical amino acids

Q3. What is the minimum number of pH buffering zones for any amino acid? **(10 points)**
(Enter a whole number.)

Q4. Indicate whether the following statements are true or false with reference to the data and graph provided above. **(40 points)**
(Mark each statement as true or false.)

A. Possible combinations of favourable dihedral angles may be larger than that of allowed dihedral angles.
B. Only the amino acids with positively-charged side chains or negatively-charged side chains can react with HCl or NaOH.
C. 1 mole of NaOH will react with 1 mole of neutral tyrosine.
D. In neutral zwitterionic lysine, the α-NH_2 group is positively charged and exists as -NH_3^+.

Other than the carboxyl and amino groups, amino acids also contain R-groups. The R-groups of amino acids are responsible for the determination of the chemical and conformational properties of a protein.

Q5. Match the following properties to the possible amino acid(s) that can have the property. The number of possible amino acids has been provided for you in the table. **(40 points)**
(Give the one-letter amino acid code. If there is more than one possible amino acid, give the codes in alphabetical order. For example, if the answer is glycine and alanine, enter AG.)

Property	**Amino Acid(s)**
Allows the resultant protein to have a high optimum temperature and temperature of denaturation *(only 1 possible amino acid)*	
Allows the formation of a tight triple helix by fitting into the tight restricted space *(only 1 possible amino acid)*	
Relatively high proportion in histones, allowing histones to form electrostatic attraction with negatively-charged DNA. *(only 3 possible amino acids)*	
Phosphorylation of this amino acid in insulin receptors is responsible for the initiation of a phosphorylation cascade *(only 1 possible amino acid)*	

Answers and Explanations

Q1.

Answer: **C**

Explanation: Glycine does have a Ramachandran plot. As it is achiral, the Ramachandran plot of glycine resembles that of L-amino acid as much as that of D-amino acid. This can also be inferred from the graph.

Q2.

Answer: **F**

Explanation: Glycine. It has the smallest R group, hydrogen, so it has the greatest number of possible permutations of dihedral angles.

Q3.

Answer: **2**

Explanation: One from the amino group, one from the carboxyl group.

Q4.

Answer: **FFFF**

A. There will be necessarily fewer favourable dihedral angles than permitted dihedral angles.
B. All amino acids have an amino group, which can react with HCl and a carboxyl group, which can react with NaOH.
C. For neutral tyrosine, the NaOH will react with both the phenolic R group and the amino group, so 2 mol of NaOH can react with neutral tyrosine.
D. The α-NH_2 group has a lower pKa than the -NH_2 in the R group so the α-NH_3^+ of the acidic form of lysine gets deprotonated first. Hence, in the neutral zwitterionic form, the α-NH_3^+ of the R group and the α-COO^- are the charged groups.

Q5.

Answer: **C, G, HKR, Y**

A. Cysteine forms covalent disulfide bonds, which are the strongest R-group interactions, hence providing the greatest stability to the tertiary structure of a protein.
B. Glycine is the smallest amino acid and hence can fit in the tight restricted space of a tropocollagen molecule.
C. These are the 3 basic positively-charged amino acids.
D. The insulin receptor is a receptor tyrosine kinase, so tyrosine is the amino acid that gets phosphorylated.

Credits

Figure 1: Dcrjsr. (2017, 28 March). *Dihedral angle*. Wikipedia. https://en.wikipedia.org/wiki/Dihedral_angle#/media/File:Protein_backbone_PhiPsiOmega_drawing.svg

Figure 2: Modified from Valiyaveetil, F.I., Sekedat, M.D., MacKinnon, R., & Muir, T.W. (2004). Glycine as a D-amino acid surrogate in the K(+)-selectivity filter. *Proceedings of the National Academy of Sciences of the United States of America, 101 49*, 17045-9. https://doi.org/10.1073/pnas.0407820101

Figure 3: Modified from Lvwarren. (2014, 12 July). *Titration Curves of 20 Amino Acids Organized by Side Chain*. Wikipedia. https://en.m.wikipedia.org/wiki/File:Titration_Curves_of_20_Amino_Acids_Organized_by_Side_Chain.png

P03: Family Guy

(200 points)

Contraceptives are methods employed to prevent pregnancies. One form of contraceptives are oral contraceptive pills for females which contain progestin and oestrogen to prevent pregnancy. While there are currently no male contraceptive pills, scientists are experimenting with a hormone-based pill called dimethandrolone undecanoate (DMAU) which was determined to be safe in a small study in 2019. This pill is shown as "E" in Figure 1. Figure 1 shows four other possible contraceptives.

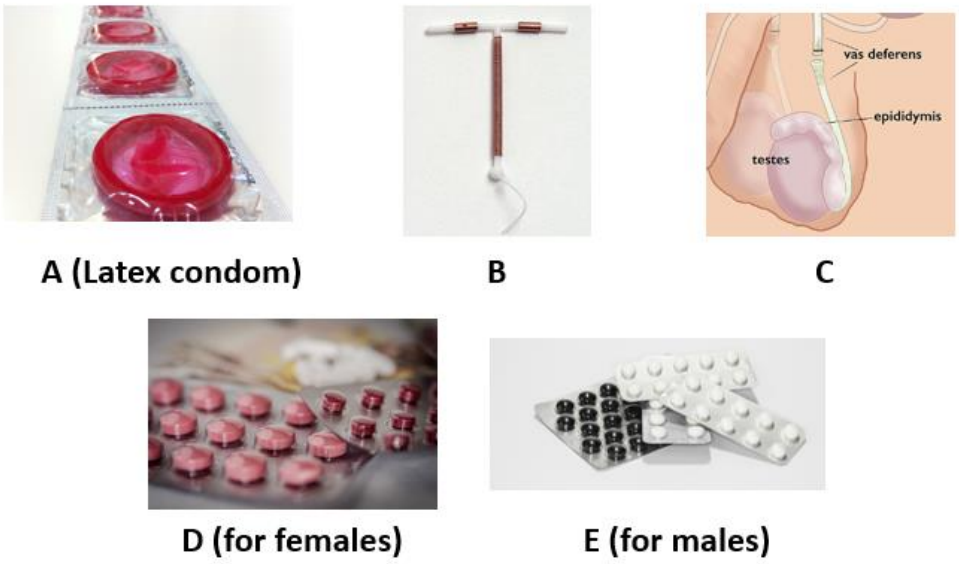


Figure 1: Possible contraceptives

You are a fertility doctor and five couples have come to you as they each need help with choosing the correct contraception. You take their history and future intentions in the table below.

Couple (F and M)	**Scenario**
Alice and Bernard	Bernard has Gonorrhoea while Alice has Syphilis. They are sure they do not want children.
Carol and Denver	A couple who recently got married. Wishes to have regular sexual intercourse for the first two years before settling down and having their own children.
Esther and Felix	A young couple who is pretty sure they do not want children. Esther has trouble consuming pills, and Felix is terrified of procedures and will not consent to any.
Giselle and Hector	An old couple who has had four children before and is sure they no longer want to have any more children.
Isabella and Joel	Isabella is allergic to latex, but Joel is not. Isabella has Wilson's disease (copper accumulation in liver) but always forgets to take her medicine. They are hoping to try for a child next year when they get a BTO flat.

Q1. You need to help match the most appropriate contraceptive method to each couple, assuming you may only use each option (A-E) once and that DMAU is available on trial to all couples and all five couples are willing to try it. **(50 points)**

(Match the correct letter to the correct row.)

Couple	**Most appropriate contraceptive (A-E)**
Alice and Bernard	
Carol and Denver	
Esther and Felix	
Giselle and Hector	
Isabella and Joel	

Another contraceptive method is the rhythm method, which makes use of the menstrual cycle to predict when the female is most fertile and to avoid sexual intercourse during then. After ejaculation, sperm remains viable in the vaginal tract for up to five days and eggs remain viable for up to 24 hours after ovulation. This forms the fertile period, during which fertilisation and hence implantation can occur.

Kimberly and Luis wish to make use of this natural contraceptive to avoid getting pregnant. Kimberly has been tracking her period for the last two months and has taken note of her last periods in the calendar below.

Week	**M**	**T**	**W**	**T**	**F**	**S**	**S**
2024 June							
Week 22						1	2
Week 23	3	4	5	6	7	8	9
Week 24	10	11	12	13	14	15	16
Week 25	17	18	19	20	21	22	23
Week 26	24	25	26	27	28	29	30
2024 July							
Week 27	1	2	3	4	5	6	7
Week 28	8	9	10	11	12	13	14
Week 29	15	16	17	18	19	20	21
Week 30	22	23	24	25	26	27	28
Week 31	29	30	31				
2024 Aug							
Week 32				1	2	3	4
Week 33	5	6	7	8	9	10	11
Week 34	12	13	14	15	16	17	18
Week 35	19	20	21	22	23	24	25
Week 36	26	27	28	29	30		

Legend: Red: Start of period | Yellow: National Day | Green: SBL Day

Q2. Assuming that Kimberly has a consistent 26-day menstrual cycle with ovulation occurring on Day 13, and considering the fertile period of Kimberly, answer the following questions. Also assume that ejaculation and ovulation occur at 0000hrs of the respective days. **(30 points)**
(Enter a number to each row.)

Question	**Answer**
When will Kimberly experience her first period in 2024 August? *(Leave your answer as DDMMYYYY.)*	
On which day should Kimbery and Luis stop having sexual intercourse in 2024 August? *(Leave your answer as DD.)*	
On which day can Kimbery and Luis resume having sexual intercourse in 2024 August? *(Leave your answer as DD.)*	

In female mammals of the subclass Theria, they do not have menstrual cycles but instead have oestrous cycles. Monoestrous species like bears only have one oestrous cycle per year, while polyoestrous species like cats have multiple oestrous cycles per year.

A distinction between the oestrous and menstrual cycle is that animals that have oetrous cycles reabsorb the endometrium if conception does not occur, in contrast to those with menstrual cycles who shed theirs via menstruation. After conception, some animals also eat their own placenta.

Unlike humans, most other species do not have menopause. Menopause is referred to as the cessation of ovulation and menstruation. After menopause, the female is no longer able to conceive and reproduce.

Q3. Based on the given preamble, indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. Monoestrous species likely lack progesterone and oestrogen while polyoestrous species likely contain progesterone and oestrogen.
B. A possible evolutionary benefit of monoestrous species is that they can be pregnant more frequently than species with a monthly menstrual cycle.
C. A possible evolutionary benefit of eating the placenta is to prevent signalling to predators that there has been a recent birth.
D. Menopause may have evolved in humans to allow a mother to stop bearing so that she can focus on taking care of her children.

Monica is a zookeeper at a zoo and needs to predict the best time to let Animal X mate. Figure 2 shows the seasonal levels of oestrogen and progesterone in Animal X. Animal X is known to be a monoestrous species.

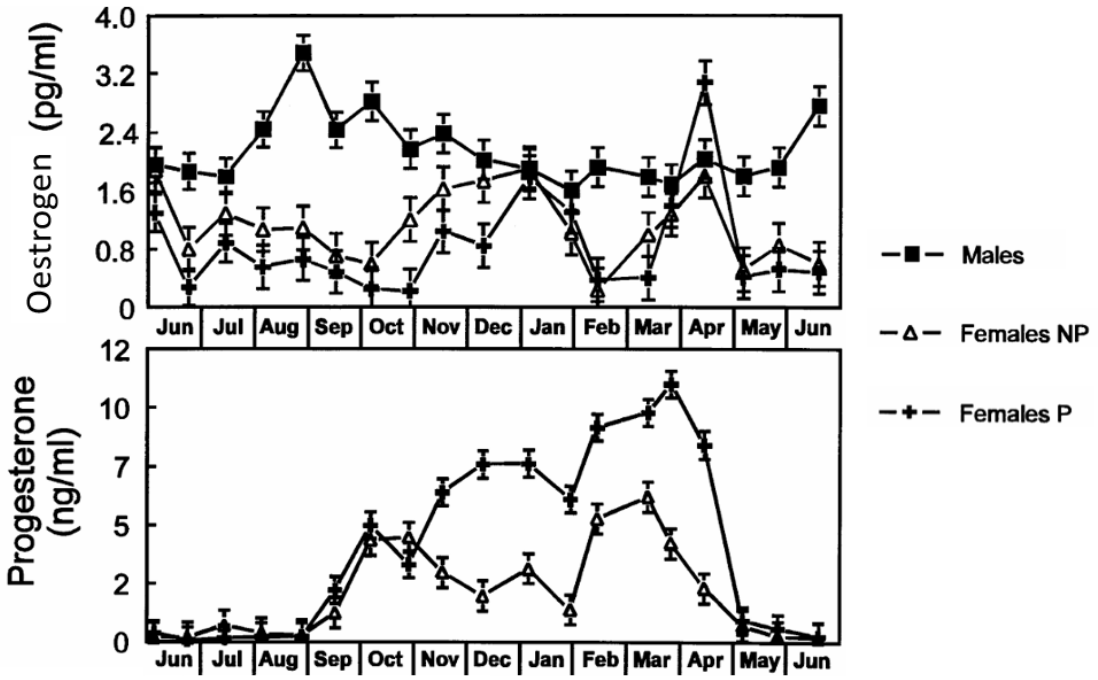


Figure 2: Seasonal changes of oestrogen and progesterone in animal X.
NP: not pregnant; P: pregnant

Q4. By comparing the changes in the graphs with that of a human female and the time of ovulation, which of the following months is the best time to let animal X mate? **(20 points)** *(Select the correct option.)*

A. January
B. April
C. July
D. November

Different species of animals have also evolved different forms of mating unlike that of humans.

- During copulation, the male honeybee's penis explodes in the female's vaginal tract. While the male subsequently dies, the exploded penis plugs and blocks the vaginal tract.
- Some male ducks have corkscrew-shaped penises and female ducks have vaginas that spiral in the opposite direction. During mating, the penis is inserted all the way to the end of the spiral vaginal tract, and semen is released.
- After sexual intercourse, female praying mantis often chew off the head of the male praying mantis, killing the praying mantis.

Q5. Based on the given preamble, indicate whether the following statements are true or false. **(40 points)**

(Mark each statement as true or false.)

A. A possible evolutionary benefit of honeybee's exploding penises is it prevents other males from mating with the female bee.
B. A possible evolutionary benefit of corkscrew-shaped penises and vaginas in ducks is that sperm will need to swim through the spiral vaginal tract to reach the eggs. Hence, stronger sperms are more likely to reach the egg faster and are selected for.
C. A possible evolutionary benefit of praying mantis eating their mate is that it prevents the male from mating with other females.
D. Male honeybees and male praying mantises likely can only mate once in their lifetime.

Oogenesis, the production of oocytes, starts in the female embryo. Primordial germ cells divide to form oogonia, which then develop to form primary oocytes. Human females are thus born with oocytes in her ovaries. During birth, she has about 1 to 2 million primary oocytes, while at puberty, she has about 300 000 oocytes. However, not all oocytes mature to become eggs. Approximately 500 oocytes fully mature between puberty and menopause, and one oocyte is released approximately every month during ovulation. Before the eggs are released, the follicles need to mature. They increase in size and swell, and they burst open during ovulation releasing one egg. After all eggs have been released over many years, menopause occurs. Menopause is defined as 12 months after a female's last period. Hence, the female is no longer able to be pregnant.

Q6. Naomi is a female with a perfect 1-month menstrual cycle and has exactly 200 oocytes which will be matured in **each** of her ovaries. She started menarche (first period) immediately after she turned age 12 and got pregnant twice. Assuming her menstrual cycle returned exactly 2 months after delivery of each of her children, calculate at which age she will reach menopause. **Round up** to the nearest whole number. **(20 points)**

(Leave your answer rounded up to the nearest whole number.)

Answers and Explanations

Q1.

Answer: **A, D, B, C, E**

Explanation: To deduce this, we must take note that each contraceptive can only be matched to one couple. Alice and Bernard both have sexually transmitted infections (STIs) and **should use a condom (A) to prevent transmission of the STIs to each other**. Isabella is allergic to latex, so condoms are out of the question. She also has Wilson's disease, which is a contraindication for copper intrauterine devices (IUDs) (B). Isabella also always forgets to take her medicine, so she should not take the contraceptive pills (D). As they wish to have children in the future, sterilisation by vasectomy (C) should not be done, **so the best option for them is DMAU pills (E).** Esther should not take the contraceptive pills as she is forgetful, and Felix rejects any procedures, so a vasectomy is out of the question. **Hence, a copper IUD (B) is the best option**. Carol and Denver cannot have sterilisation as they want children in the future, **so they should use the female contraceptive pills (D)**. **The best contraceptive for Giselle and Hector is sterilisation (C)** as they are an old couple who are sure they do not want any more children.

Q2.

Answer: **04082024, 12, 17**

Explanation: Kimberly's first period in August 2024 will be 26 days after 9 July 2024, which is 4 August 2024 (Day 1). With a 26-day menstrual cycle, Kimberly should ovulate on Day 13, which would be 12 days after 4 August 2024, which is 16 August 2024. As stated in the preamble that sperm remains viable for up to five days in the vaginal tract, Kimberly should stop sexual intercourse 4 days before 16 August 2024, which is 12 August (as we assume ejaculation and ovulation occur at 0000hrs), and resume 24 hours after 16 August 2024, which is 0000hrs of 17 August 2024. (11 August and 18 August were also accepted respectively.)

Q3.

Answer: **FFTT**

Explanation:

A. This is not true and can be seen in Figure 2. Both oestrogen and progesterone fluctuate naturally to allow for the oestrous cycle to occur.
B. Monoestrous species can only be pregnant once a year, as opposed to polyoestrous species which can be pregnant more than once a year.
C. The placenta can signal to predators that a birth has recently taken place. As giving birth takes a toll on the female, it signals to the predator that there is a weak female prey available to be eaten. Hence, animals may eat the placenta to prevent signalling to the predators.
D. It is possible as humans are K-selected species and focus on developing the children as opposed to r-selected species which give birth to large numbers of offspring in hopes that at least several will survive.

Q4.

Answer: **C**

Explanation:

Looking at the human female menstrual cycle, the best time to mate is ovulation which occurs before the rise of progesterone and in between the peaks of oestrogen. Moreover, we want to avoid times where progesterone is higher as this will result in the formation of the mucous plug which plugs the cervix, preventing the sperm from entering. Another way we can deduce is to see the times where the hormone levels of pregnant females deviate from those of non-pregnant females, as that implies that the female can only get pregnant during those times, hinting that mating should have occurred before then.

From this we can deduce that mating cannot occur in November, January, or April where progesterone levels are higher. Hence, the best time must be July. Indeed, the true best mating time period of this monoestrous species is between July and August, where oestrogen levels are not too steep and progesterone levels are at a minimum.

Q5.

Answer: **TFFT**

Explanation:

A. Plugging the female vaginal tract prevents other males from depositing sperm into the vaginal tract, hence ensuring that any offspring will belong to that male and not any other males.
B. The preamble has stated that the penis goes all the way through the corkscrew-shaped vaginal tract and the sperm is released at the end. Hence, the sperm do not need to swim along the vaginal tract, so it is unlikely that that is the evolutionary benefit.
C. It does not make any sense to prevent the male from with other females if the female has already mated with the male. A more reasonable explanation would be as a food source providing nutrients for the conceiving mother.
D. As male honeybees lose their penises and die after mating, and male praying mantises are eaten after mating, they are no longer able to mate after mating once.

Q6.

Answer: **49**

Explanation:

With 200 oocytes in each ovary, she will have 400 oocytes to release before menopause. Two of them will be released during ovulation and fertilised for pregnancy. Hence, 398 oocytes will be released accounting for 398 menstrual cycles. Since she releases one oocyte each month, she will take 33 years and 2 months to release all 398 oocytes. This puts her at the end of Age 45 Month 2.

She also became pregnant twice. Pregnancy on average takes 38 weeks or 9.5 months from the point of ovulation, which occurs at the middle of the menstrual cycle (Jukic, A. M., Baird, D. D., Weinberg, C. R., McConnaughey, D. R., & Wilcox, A. J. (2013). Length of human pregnancy and contributors to its natural variation. *Human reproduction (Oxford, England)*, *28*(10), 2848–2855. https://doi.org/10.1093/humrep/det297).

For the first pregnancy, ovulation would occur in the middle of Age 45 Month 3. Adding 9.5 months to this, we will get the end of Age 46 Month 1. Her menstrual cycle will return 2 months post-partum, so her menstrual cycle will resume at the end of Age 46 Month 3. Similarly, for the pregnancy, ovulation will occur in the middle of Age 46 Month 4, and the child will be born at the end of Age 47 Month 2. Her menstrual cycle will then resume at the end of Age 47 Month 4.

Since menopause is one year after the last menstruation, menopause will start at the end Age 48 Month 4. Rounding up, we get 49 years of age.

Note: There is some ambiguity intentionally present in the question which would require participants to make certain fair assumptions. The question posed that Naomi has a perfect "1-month" menstrual cycle so as to allow calculations to be easier by eliminating the differences in months with 30 days and 31 days.

There is also some ambiguity as to how a "month" is defined. While it is often regarded that pregnancy takes 10 months, this operates under the assumption that one month is 28 days. This is in general not true, and on average one month is $\frac{52}{12} = \mathbf{4.3333}\ \boldsymbol{weeks}$*. This means that pregnancy only takes* $\frac{38}{4.33} = \mathbf{8.77}\ \boldsymbol{weeks}$*.*

However, even if we assume that pregnancy is 8.77 weeks, we will still get the final answer of 49 years. This is because the first child will be born slightly past Age 45 Month 12, and the second child will be born slightly past Age 46 Month 12, so menopause will be slightly past Age 48 Month 2.

Moreover, even if we erroneously assumed that pregnancy of 8.77 weeks starts at the start of the menstrual/ovarian cycle, it would only shave off half a month for each pregnancy, leaving us with menopause starting slightly past Age 48 Month 1.

Therefore, regardless of which assumption(s) was made, the final answer would still be 49 years of age, as the question requires us to round ***up*** *our answer.*

Credits

Figure 1: Images are taken and modified from:

IUD Device: LeiaWonder. (2012, July 24). *A common copper IUD (Paragard) with scale markings.* Wikipedia. https://en.wikipedia.org/wiki/Intrauterine_device#/media/File:IUD_with_scale.jpg

Vasectomy: Timdwilliamson. (2019, June 21). *Open-ended vasectomy*. Wikipedia. https://en.wikipedia.org/wiki/Vasectomy#/media/File:Open_Vasectomy_.jpeg

Figure 2: Bubenik, G. A., Schams, D., White, R. J., Rowell, J., Blake, J., & Bartos, L. (1997). Seasonal levels of reproductive hormones and their relationship to the antler cycle of male and female reindeer (Rangifer tarandus). *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology*, *116*(2), 269–277. https://doi.org/10.1016/s0305-0491(97)00183-1

Content reference: Jukic, A. M., Baird, D. D., Weinberg, C. R., McConnaughey, D. R., & Wilcox, A. J. (2013). Length of human pregnancy and contributors to its natural variation. *Human reproduction (Oxford, England)*, *28*(10), 2848–2855. https://doi.org/10.1093/humrep/det297

P04: Feathers and Mr. Birdy

(100 points)

Most bird species dislike wind and rain conditions and often migrate to other warmer areas during winter. According to National Geographic, approximately 40% of the birds in the world migrate to warmer areas which have more food. With Singapore's warm weather all year round, Singapore is a hotspot for many of these birds.

Bird Paradise was opened on 8 May 2023 which replaced the Jurong Bird Park as an aviary in Singapore. As a cytogeneticist working there, you are investigating a case of aneuploidy in one of the birds called Feathers. The normal karyotype of a bird from the same species is seen in Figure 1.

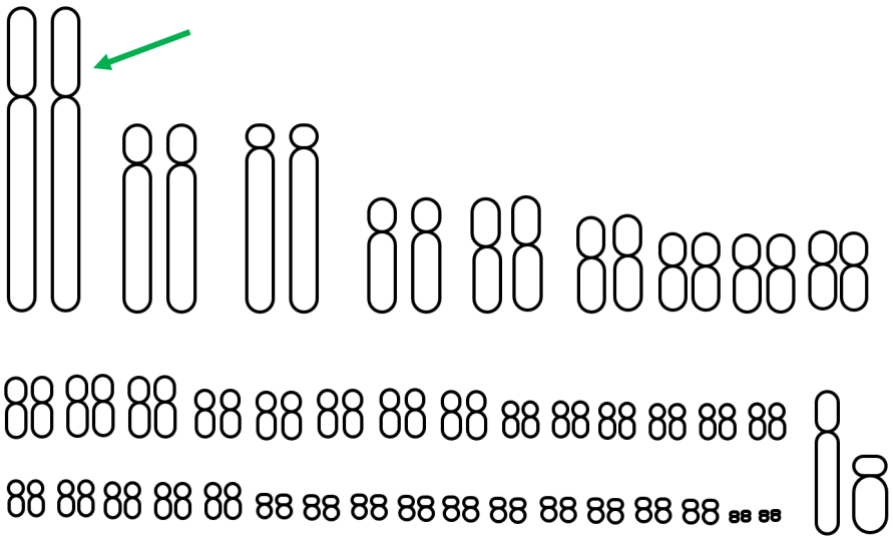


Figure 1: Normal Karyotype of species of Feathers. The green arrow indicates Chromosome 1.

Q1. Indicate whether the following statements regarding this species are true or false. **(40 points)** *(Mark each statement as true or false.)*

A. This karyotype could have been taken during metaphase of mitosis using colchicine.
B. Chromosome 1 is telocentric.
C. The karyotype was likely taken from a male.
D. The normal gamete of this species will contain 40 autosomal chromosomes.

Feathers has a trisomy of chromosome 1. This implies that at least one gamete had mutations. Figure 2 shows the staining pattern of chromosome 1 for Feathers and its parents.

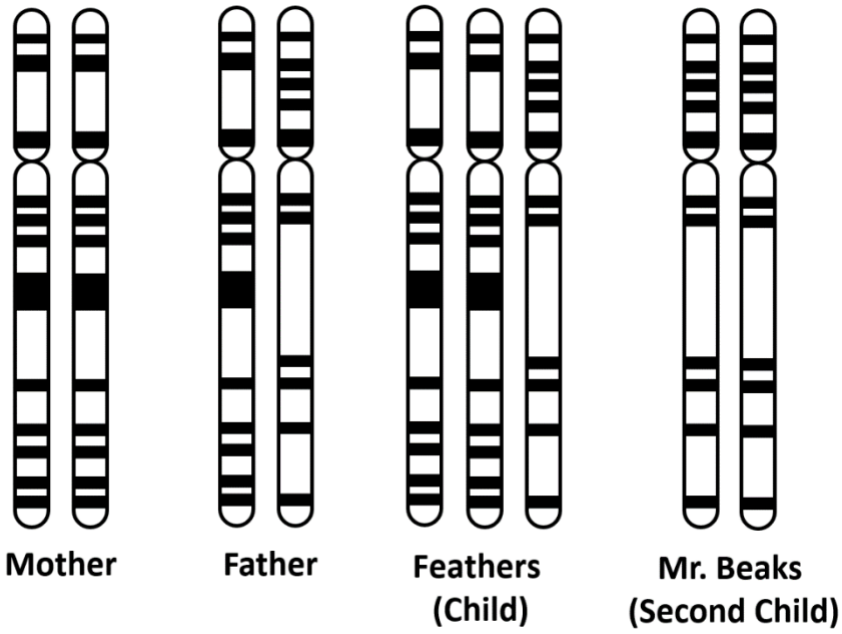


Figure 2: Staining pattern of chromosome 1 of Feathers, Mr. Beaks and their parents

Q2. Which of the following **could not** have happened during gametogenesis to give rise to Feather's trisomy 1? **(30 points)**
(Select all correct options.)

A. Non-disjunction during Meiosis I during spermatogenesis.
B. Non-disjunction during Meiosis II during spermatogenesis.
C. Non-disjunction during Meiosis I during oogenesis.
D. Non-disjunction during Meiosis II during oogenesis.

Mr. Beaks is Feathers's younger brother from the same parents. As a cytogeneticist, you noticed that the staining pattern of chromosome 1 of Mr. Beaks is unusual. You hypothesised that it may have arisen due to non-disjunction during gametogenesis.

Q3. Which of the following **could not** have happened during gametogenesis to give rise to Mr. Beaks' chromosome 1 staining pattern? **(30 points)**
(Select all correct options.)

A. Non-disjunction during Meiosis I during spermatogenesis.
B. Non-disjunction during Meiosis II during spermatogenesis.
C. Non-disjunction during Meiosis I during oogenesis.
D. Non-disjunction during Meiosis II during oogenesis.

Answers and Explanations

Q1.

Answer: **FFFT**

Explanation:

A. Since the chromosomes are visible, the chromatin has already been tightened to chromosomes, so prophase must already have occurred, and the karyotype must have been taken before the chromosome is loosened into chromatin. All chromosomes only contain one sister chromatid, so this must have occurred after anaphase where the two sister chromatids are pulled apart forming two daughter chromosomes each with one sister chromatid. Since each cell only contained two copies of each homologue as opposed to four, it is likely the two sets of chromosomes have already been separated, so this karyotype is likely taken during telophase before the chromosomes are loosened. Metaphase chromosomes would present as two sister chromatids attached at one centromere for each chromosome.

B. Chromosome 1 is not telocentric as telocentric chromosomes have the centromere at the end of the chromatid. Hence, it should appear as one chromatid with a centromere at the end in the karyotype. This chromosome is submetacentric.

C. In birds, males are the homogametic sex while females are the heterogametic sex. Hence, males have two Z chromosomes (ZZ) while females have one Z and one W chromosome (ZW). Since the two sex chromosomes are differently shaped in the karyotype, the chromosomes are different so the bird is of the heterogametic sex. Hence, the bird is a female.

D. We can count 40 pairs of autosomes and one pair of allosomes (Sex chromosomes). Hence, the normal gamete will contain 40 autosomes and one allosome due to meiosis halving the ploidy number.

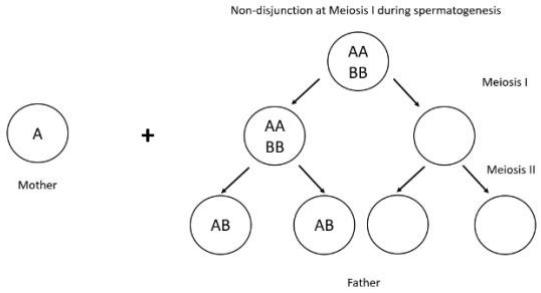
Q2.

Answer: **B**

Explanation: We will label the chromosome on the left of the father in Figure 2 as A and that on the right as B. Feathers has three chromosomes, thus one parent passed on one chromosome while the other passed on two chromosomes. Feathers received Chromosome B which could only have been from the father.

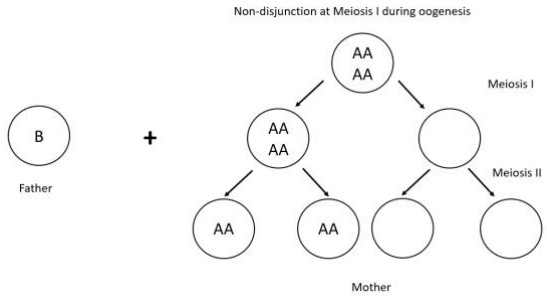
Analysing each scenario:

A. This could have led to non-disjunction of Chromosome 1, so the gamete will each contain Chromosome A and B. Fusion of this sperm with a normal egg will yield Feather's karyotype.

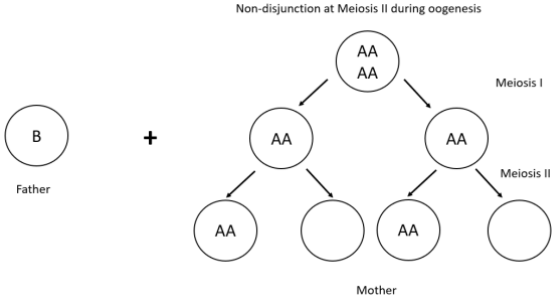


B. This is not possible as Feathers must get Chromosome B from the father. However, if non-disjunction had occurred during Meiosis II of spermatogenesis, then the sperm will contain two copies of Chromosome B, so Feathers would thus have two copies of Chromosome 1. Hence, regardless of the mother's egg, non-disjunction during Meiosis II of spermatogenesis cannot have occurred.

C. Non-disjunction at Meiosis I during oogenesis will result in eggs with two copies of Chromosome A, which can fuse with a normal sperm with one copy of Chromosome B to form Feathers.



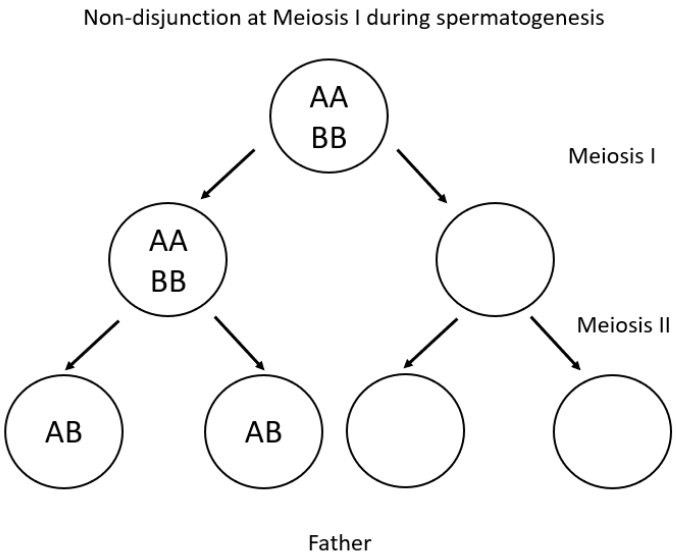
D. Non-disjunction at Meiosis II during oogenesis will result in eggs with two copies of Chromosome A, which can fuse with a normal sperm with one copy of Chromosome B to form Feathers.



Q3.

Answer: **A**

Explanation: We notice that Mr. Beaks has two copies of Chromosome B, thus a mutation must have occurred in the sperm of the father to give rise to a duplicated Chromosome 1. At the same time, the egg must also not have any Chromosome 1 so that the mother does not pass on any Chromosome A to Mr. Beaks. Since either non-disjunction during Meiosis I or Meiosis II of oogenesis can lead to an egg with no Chromosome 1, both of them are possible. However, since no Chromosome A is present in Mr. Beaks and consequently in the sperm, non-disjunction cannot have occurred in Meiosis I of spermatogenesis as it will result in Chromosome A being passed on to the sperm with Chromosome B. Non-disjunction must have occurred in Meiosis II of spermatogenesis in the cell containing two copies of Chromosome B, thus the sperm now contains two copies of Chromosome B.



P05: Gummyfish

(100 points)

Gummyfishes used to thrive in aquatic environments several years ago. As its population rose dramatically and resources in the water depleted, some individuals eventually evolved to move to land, where there would be less competition.

Q1. Several traits have been identified in various present-day descendants of gummyfishes found in aquatic and terrestrial environments. These traits may have different benefits for the gummyfishes. Match each trait to how it benefits the gummyfishes on land using the numbers 1-4. **(60 points)**
(Enter a number to each row.)

1. Aids respiration on land
2. Aids movement on land
3. Other benefit on land
4. No benefit on land

Trait	**Benefit (1-4)**
Nostrils	
High refractive index of lens	
Moist skin	
Presence of extraembryonic membranes	
Connection of pelvis to ribs	
Broad and flattened limbs	

Due to the stark differences in adaptations necessary to thrive on land as opposed to water, the transition from a fully aquatic species to a fully terrestrial one cannot occur directly without an intermediate state, which is semiaquatic. The interconversion between the semiaquatic state with either the terrestrial or aquatic state is considered as **one** distinct evolutionary event. Figure 1 shows a phylogenetic tree of the descendants of gummyfishes.

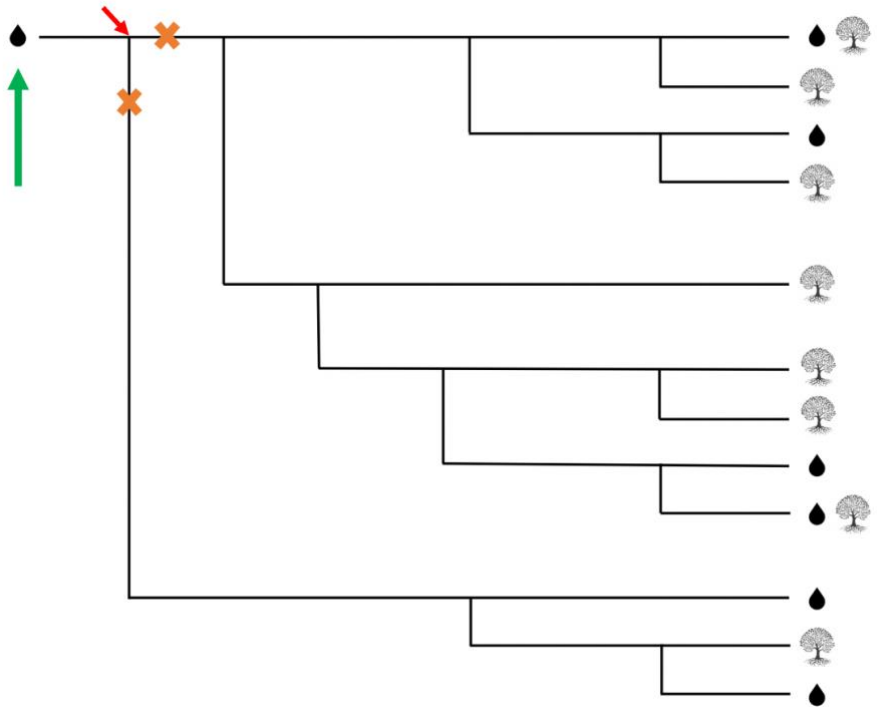


Figure 1: Phylogenetic tree of the descendants of gummyfishes. Water droplets indicate fully aquatic species, trees indicate fully terrestrial species, and the presence of both symbols indicate semiaquatic species.

The ancestor of all the descendants is marked by the green arrow. This ancestor is a fully aquatic species. Over long periods of time, mutations can accumulate in the genome of the ancestors which changes the habitat type of the species. However, mutations are rare so such changes do not occur very frequently.

At the node indicated by the red arrow, the tree splits into two. If no changes in habitat type had occurred before this node, then the ancestors that go into the two lineages will still have the same habitat type, as seen by the orange crosses.

We can see that there are several changes in the habitat type of the descendants. We would like to see what the minimum number of changes in habitat type is that could have occurred to give rise to this phylogenetic tree. Such a tree is called the most parsimonious phylogenetic tree.

Occam's razor is a principle used in phylogenetics to find the most parsimonious phylogenetic tree. Occam's razor states that if hypotheses have equal explanatory powers, the one requiring the fewest assumptions should be the preferred hypothesis. Hence, with all other things being equal, the

phylogenetic tree which requires the fewest number of changes in habitat types is the best hypothesis and can be used as the most parsimonious tree.

Using the tree in Figure 1, consider at which points of the tree changes in habitat types could have occurred. Remember that any change in the habitat type would mean that all descendants from that ancestor will have that new habitat type, unless another change occurred to change the habitat type.

Q2. What is the minimum number of evolutionary events required to account for the phylogenetic tree presented? Note that fully aquatic, semiaquatic, and fully terrestrial habitats are considered distinct habitats. **(30 points)**
(Enter your answer as a whole number.)

Q3. Which term correctly describes the group formed by all the fully aquatic species? **(10 points)**
(Select the correct option.)

A. Monophyletic
B. Paraphyletic
C. Polyphyletic
D. None of the above

Answers and Explanations

Q1.

Answer: **1, 4, 1, 3, 2, 4**

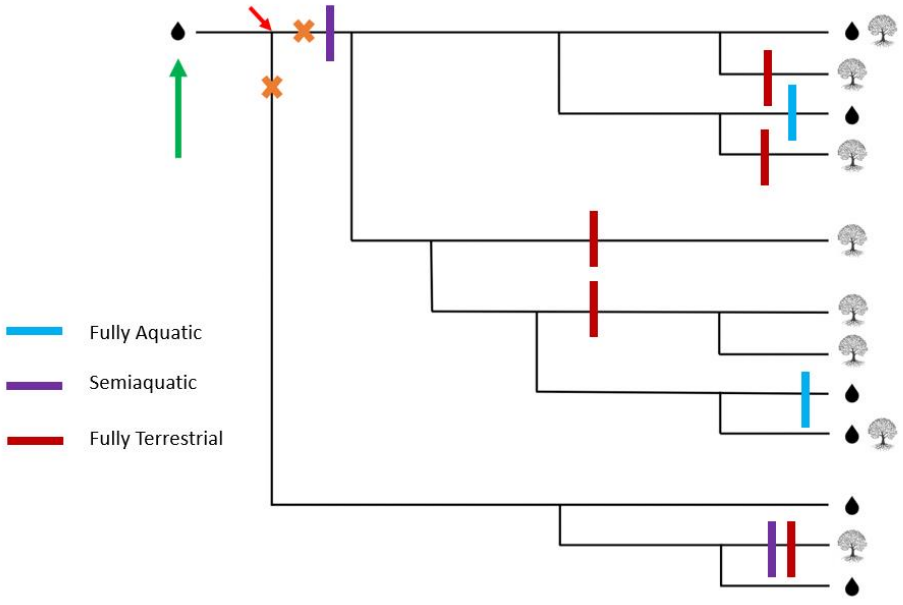
Explanation:

A. Nostrils allow the animal to take on gaseous oxygen to breathe on land.
B. Land animals should have a lower refractive index, as we can see that the refractive index of air (1.00) is less than that of air (1.33). This is likely an evolutionary relic from gummyfish ancestors in the aquatic environment as refraction is more important when the refractive index of the medium i.e. the water is closer to that of the lens.
C. Moist skin allows for gaseous exchange on land like amphibians.
D. Allows for shelled eggs (amniotes).
E. Allows for the formation of a skeleton which connects the upper body to the lower body allowing for movement on land.
F. These are similar to flippers and would aid in the water. Such limbs would make movement on land very awkward.

Q2.

Answer: **9**

Explanation: Each evolutionary change can be seen in the diagram below. Remember that you must pass through a semiaquatic state to get from the aquatic to terrestrial state.



Q3.

Answer: **C**

Explanation: All aquatic species are several different descendants from different ancestors, and their common ancestor is not included.

P06: Modifications

(120 points)

A method of transcriptional regulation is the formation of euchromatin and heterochromatin. The DNA in cells is present in the form of chromatin. Loosely-packed chromatin is called euchromatin, while tightly-wound chromatin around histones is called heterochromatin. Being more tightly packed, heterochromatin is less exposed and hence less accessible to the action of transcription by DNA polymerases.

Figure 1 shows the ultrastructure of a cell.

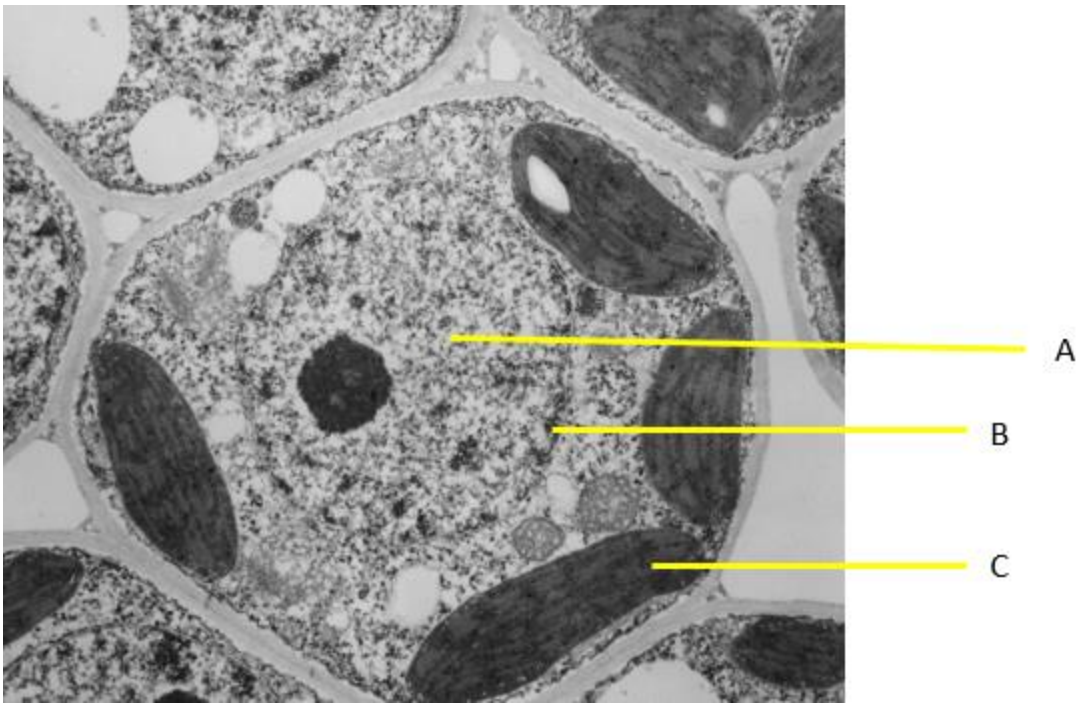


Figure 1: Ultrastructure of cell

It has been determined that either gene D or E is transcriptionally active in the cell in Figure 1. To determine which gene is transcriptionally active and hence the DNA is less tightly wound, you perform an experiment. You extracted the DNA from the cell and treated them with a non-specific endonuclease. Such an endonuclease breaks the phosphodiester bonds randomly in the DNA hence cleaving the phosphate backbone into two. Hence, there is a chance that the endonuclease will cleave the gene, preventing probes from being able to bind to it. **The entire sequence of the gene must be intact for the antibodies to bind to it.**

You then isolated the cleaved DNA and incubated them with an excess of radioactive probes specific for a 20-nucleotide long sequence in either gene D or gene E. The results are seen in Figure 2.

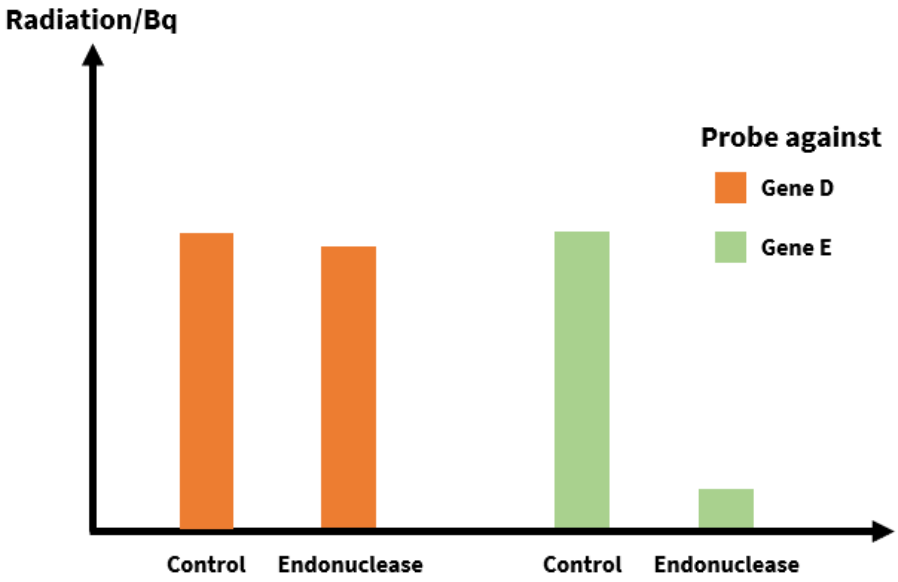


Figure 2: Radiation after incubation with probes specific to different genes. Control: Absence of endonuclease. Endonuclease: Presence of endonuclease. Endonuclease was removed before addition of probes.

Q1. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The cell in Figure 1 is a plant cell.
B. Structure A is heterochromatin and Structure B is euchromatin.
C. Structure C is the mitochondria.
D. Gene D is transcriptionally active in the cell.

Another form of modification occurs after the polypeptide chain is synthesised. Post-translational modifications occur to prepare the protein for its functional role in or out of the cell. Figure 3 shows three examples of post-translational modification-regulation of RNA-binding proteins.

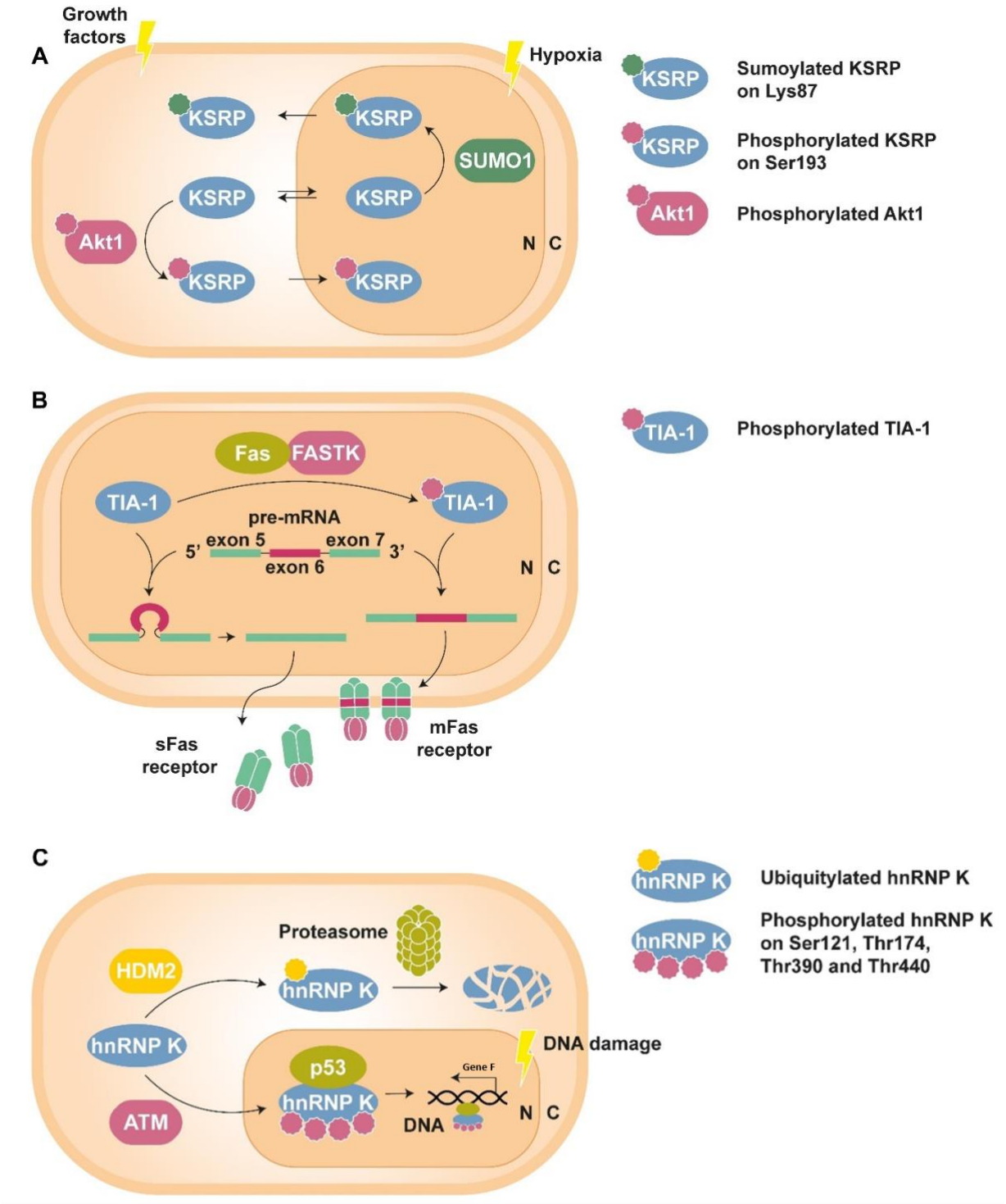


Figure 3: Post-translational modification-regulation of RNA-binding. N: Nucleus. C: Cytoplasm. Lightning symbols indicate the conditions that will stimulate such a pathway in the cell. (A) KSRP and SUMO1 each perform specific functions in the nucleus and the cytoplasm. (B) mFas plays an important role in extrinsic apoptosis signalling pathways, while sFas blocks apoptosis. (C) hnRNP K binds to HDM2 under standard conditions.

Q2. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. KSRP is able to diffuse freely via simple diffusion from the nucleus to the cytoplasm and back.
B. Phosphorylated KSRP may upregulate genes involved in growth.
C. Figure 3B shows alternative splicing.
D. The translated region of exon 6 in figure 3B likely contains many amino acid residues with hydrophilic R-groups.

Q3. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. Phosphorylation of TIA-1 by FASTK is likely overactive in cancer cells.
B. Ubiquitylation of hnRNP K is required for its degradation by the proteasome.
C. hnRNP K usually binds to p53 under standard conditions.
D. In cancer cells, there is more likely a gain-of-function mutation than a loss-of-function mutation in gene F.

Answers and Explanations

Q1.

Answer: **TFFF**

Explanation:

A. The presence of the cellulose cell wall and chloroplasts (C) imply that it is a plant cell.
B. A is euchromatin as it is less densely packed and B is heterochromatin as it is more darkly stained and is hence more compact.
C. Structure C is the chloroplast as the thylakoid membranes can be seen.
D. It is Gene E that is transcriptionally active. The gene that is transcriptionally active will exist as euchromatin and will hence be less tightly packed. Hence, the gene is more accessible for the endonuclease to cut the phosphodiester bond in the gene. As the preamble states that the entire gene must be present for the probes to bind to it, less probes can bind to the DNA as the gene is no longer intact after being cut by the endonuclease, hence the radioactivity measured will be lower, as seen in Gene E.

Q2.

Answer: **FTTF**

Explanation:

A. KSRP is a very large protein that cannot simply diffuse through the cell membrane. We see that KSRP has at least 193 amino acids as the 193th amino acid is serine and can be phosphorylated. Large proteins like KSRP would require a transport protein to allow it diffuse through the cell membrane and thus cannot diffuse through the small transient pores of the cell membrane via simple diffusion.
B. In the presence of growth factors, KSRP is phosphorylated and enters the nucleus. Hence, it is likely that phosphorylated KSRP acts in the pathway to activate genes involved in growth in response to growth factors.
C. Figure 3B shows two different mature mRNAs and hence two different proteins, sFas and mFas receptors, produced from the same pre-mRNA. Hence, alternative splicing is in action.
D. Exon 6 is represented as fuchsia in Figure 3B and is seen in the mFas receptor and not the sFas receptor. Hence, exon 6 likely codes for proteins involved in the embedding of the receptor to the cell membrane, hence the loss of exon 6 results in the sFas receptor which is not embedded but is released into the extracellular environment. Exon 6 hence likely contains amino acid residues

with hydrophobic R-groups hence allowing them to interact with the non-polar hydrophobic hydrocarbon chains of the phospholipids and be embedded in the membrane.

Q3.

Answer: **FTFF**

Explanation:

A. Phosphorylated TIA-1 induces the formation of the mFas receptor which aids in apoptosis signalling pathways. Since a hallmark of cancer cells is the ability to evade apoptosis, it is likely that there are lower levels of mFas receptors in cancer cells, hence phosphorylation of TIA-1 is likely lower.
B. Ubiquitylation of hnRNP K acts as a signal to be digested by enzymes in the proteasome.
C. Under standard conditions, hnRNP K binds to HDM2 for degradation, hence less is available for binding to p53.
D. Since DNA damage induces the transcription of gene F, it is likely that gene F is a tumour suppresor gene. In cancer cells, tumour suppresor genes have loss-of-function mutations which causes them to be unable to perform their roles such as cell cycle arrest, leading to the dysregulation of the cell cycle. This is in contrast to gain-of-function mutations in proto-oncogenes forming oncogenes.

Credits

Figure 1: *TEM of Plant Cell*. Tem of Plant Cell. (n.d.). https://www.cas.miamioh.edu/~meicenrd/anatomy/Ch2_Ultrastructure/Tempcell.htm

Figure 3: Velázquez-Cruz, A., Baños-Jaime, B., Díaz-Quintana, A., De la Rosa, M. A., & Díaz-Moreno, I. (2021, March 22). *Post-translational control of RNA-binding proteins and disease-related dysregulation*. Frontiers. https://www.frontiersin.org/journals/molecular-biosciences/articles/10.3389/fmolb.2021.658852/full

P07: The Squarepants Extended Family

(160 points)

Generally speaking, sponges (phylum Porifera) are considered the simplest of animals. Unlike other animals, they do not have separate germ layers and do not have any true tissues. Most, although not all, sponges are also asymmetrical, while most other animals possess radial symmetry or bilateral symmetry.

The following are examples of asymmetry and two types of symmetry.

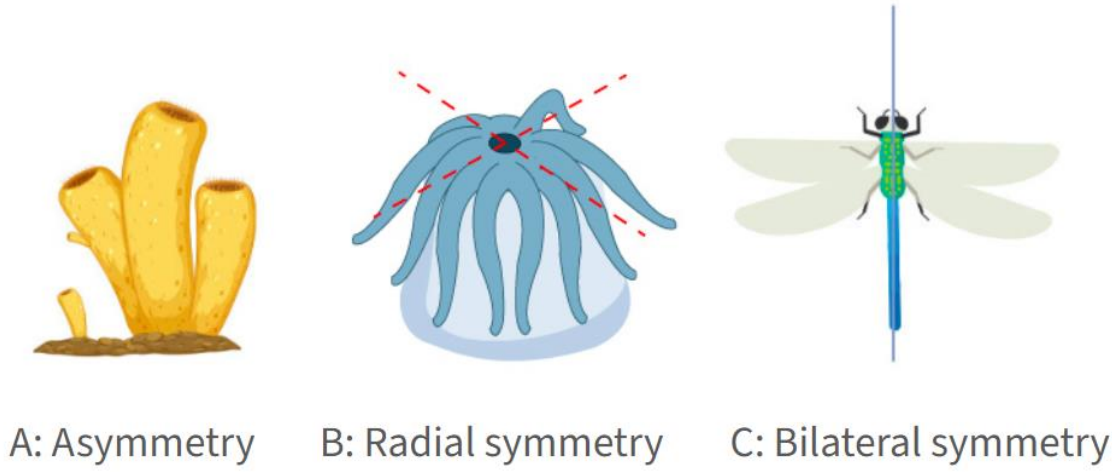


Figure 1: Examples of symmetry types

In Figure 1, Animal A has **no symmetry** or **is asymmetrical**.

Animal B is **radially symmetrical** because it has many different planes of symmetry, which can divide the body into many (roughly) equal parts around a centre from the top-down view. Two specific types of radial symmetry include **biradial symmetry** (commonly exhibited by ctenophores) and **pentaradial symmetry** (commonly exhibited by echinoderms), which means that the body can be divided into two and five equal parts around a centre from the top-down view respectively.

Animal C is **bilaterally symmetrical** because it has one plane of symmetry dividing the body into two (roughly) equal parts.

Figure 2 shows several animals with different types of symmetry.

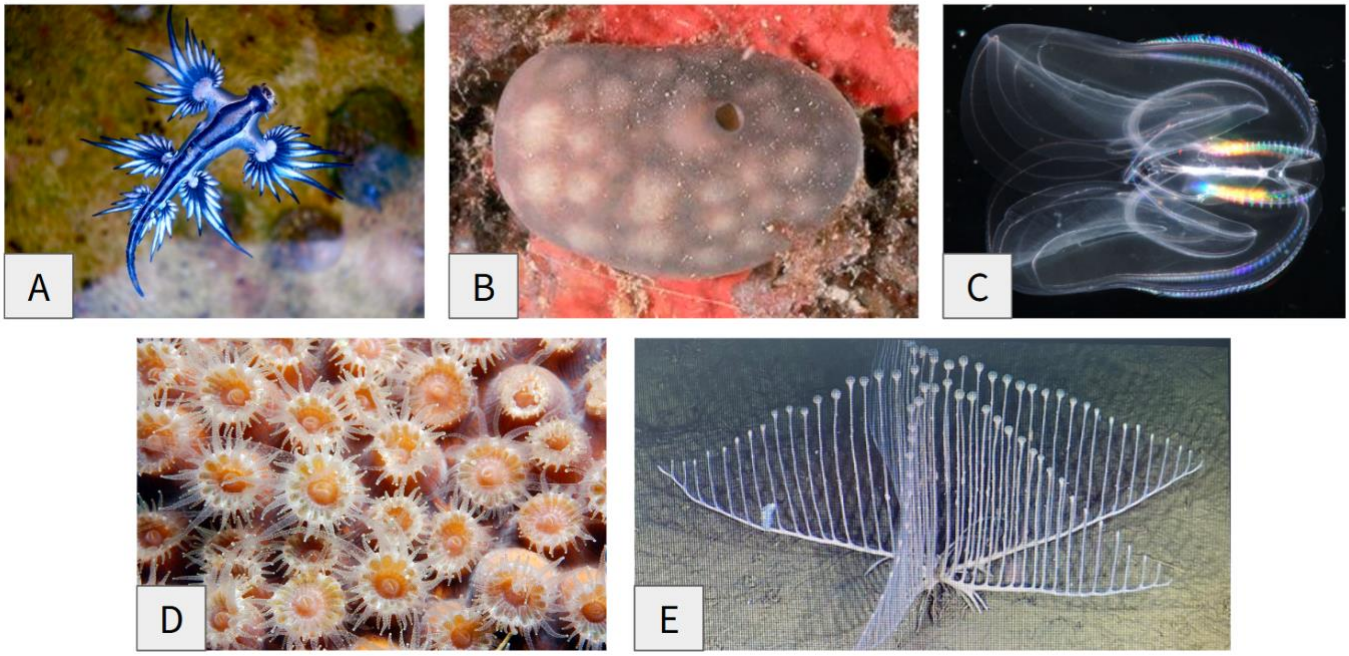


Figure 2: Various sea creatures

Q1. Match the animals to their respective type of symmetry. Each type of symmetry will only correspond to one letter. **(50 points)**

(Match the correct letter to the correct row.)

Symmetry	**Letter**
Asymmetry (no symmetry)	
Radial Symmetry (not pentaradial or biradial)	
Biradial Symmetry	
Pentaradial Symmetry	
Bilateral Symmetry	

Sponge Anatomy

Most sponges are filter feeders, with their water filtration system as shown in Figure 3.

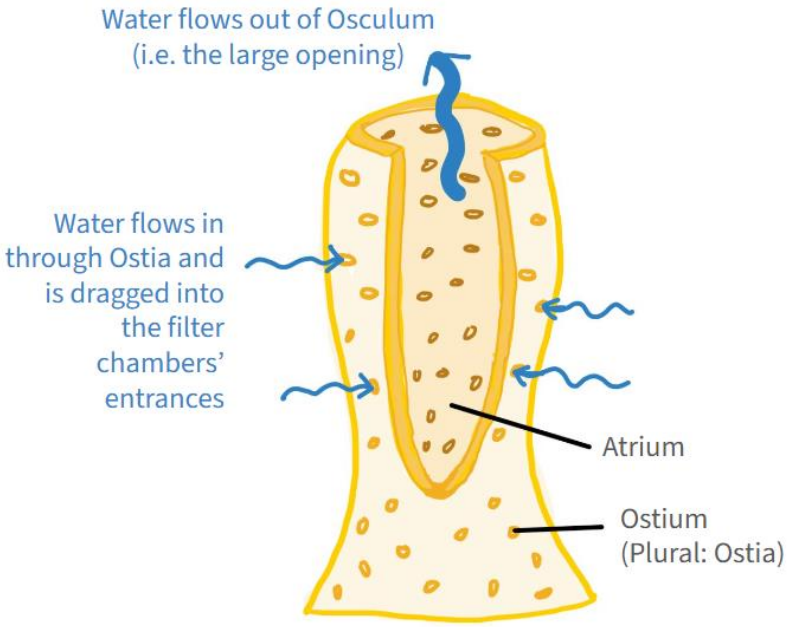


Figure 3: Structure of a sponge

Filter-feeding sponges use this filtration system to capture bacteria and other microorganisms, which are phagocytosed by amoeboid cells.

Carnivorous sponges, on the other hand, often have a different feeding mechanism that does not rely on these filters. The Ping Pong Tree Sponge (Figure 4) is a unique carnivorous sponge.

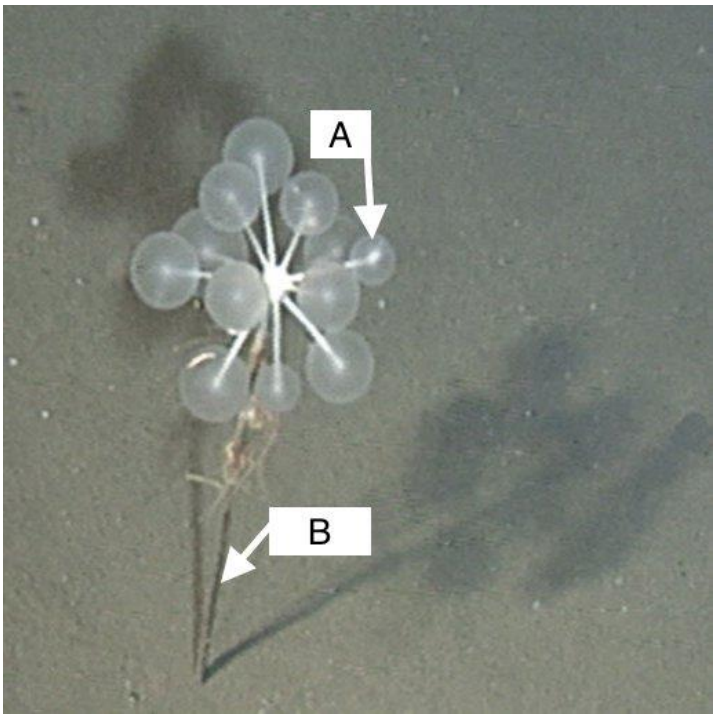


Figure 4: Ping Pong Tree Sponge (*Chondrocladia lampadiglobus*)

Q2. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. A and B are composed of different tissue types.
B. Unlike most types of sponges, the Ping Pong tree sponge (*Chondrocladia lampadiglobus*) exhibits radial symmetry.
C. After the Ping Pong Tree Sponge (*Chondrocladia lampadiglobus*) traps its prey on the surface of A, the prey is enveloped to enter the sponge's digestive cavity to be digested by enzymes secreted by the sponge's cells.
D. Unlike most carnivorous sponges, the water filtration system in the Ping Pong tree sponge (*Chondrocladia lampadiglobus*) is still present as it is required to inflate structure A.

Classification

The sister group to the Kingdom *Animalia* are a group of organisms known as "choanoflagellates". An example of a choanoflagellate is shown below in Figure 4.

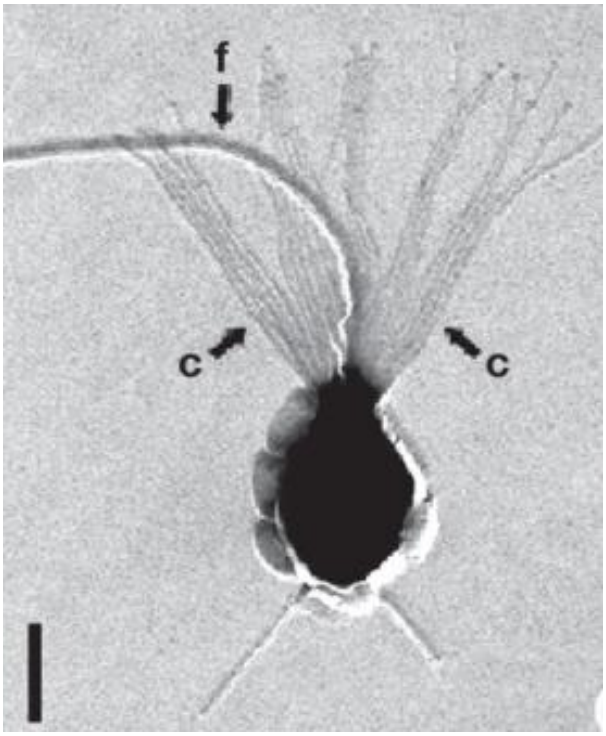


Figure 5: Choanoflagellate *Monosiga ovata*

Figure 6 shows the anatomy of a sponge along with several labelled cells.

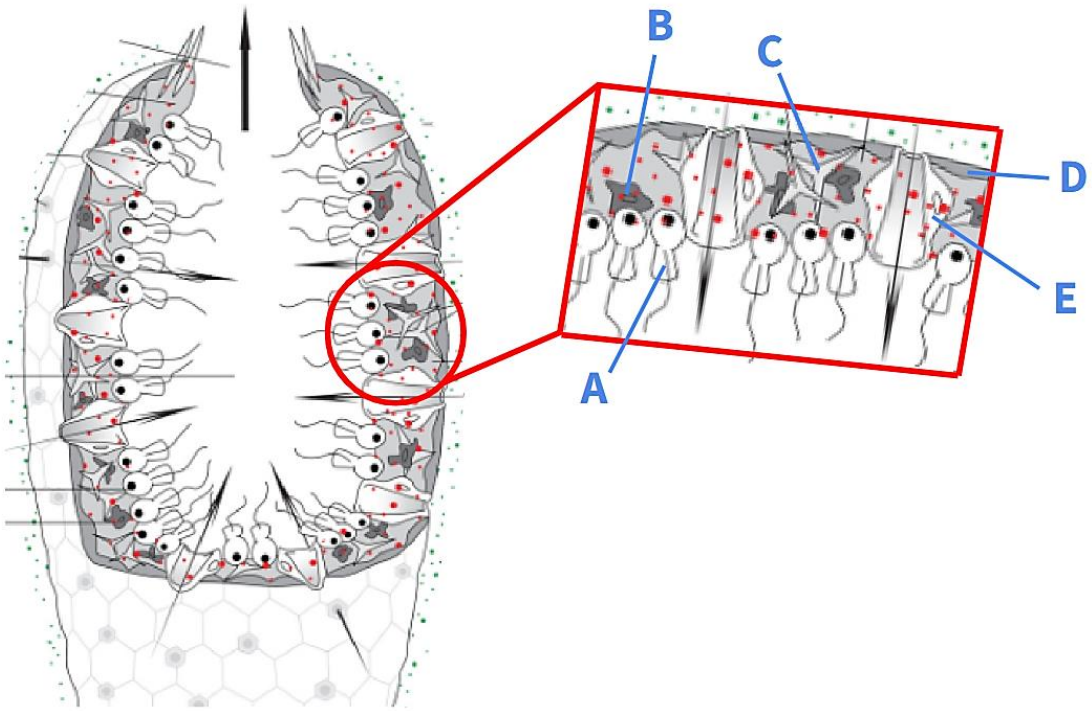


Figure 6: Anatomy of a Sponge

Q3. Which of the following cells is likely homologous to choanoflagellates? **(10 points)**
(Select the correct option.)

A. A
B. B
C. C
D. D
E. E

Table 1 lists the various traits of the phyla Porifera, Cnidaria, Ctenophora, and the Clade Bilateria.

Table 1: Different traits of phyla Porifera, Cnidaria, Ctenophora, and Clade Bilateria

	Porifera	**Cnidaria**	**Ctenophora**	**Bilateria**
Muscles	Absent	Present	Present	Present
Sensory Organs	Absent	Present	Present	Present
Hox genes	Absent	Present	Absent	Present
Digestive organs	Absent	Absent	Absent	Present
MicroRNA	Present	Present	Absent	Present

Most popular modern classifications tend to place *Porifera* as the sister group to all other clades shown above *(Cnidaria, Ctenophora, Bilateria).* However, some hypotheses place *Ctenophora* as the sister group instead.

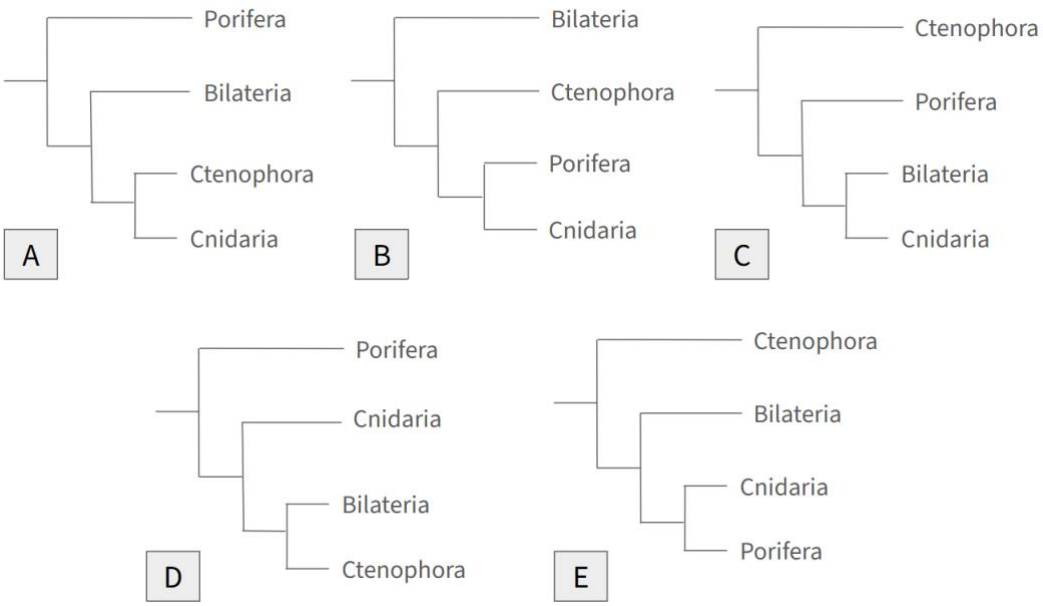


Figure 7: Hypotheses on phylogenetic classification of phyla within *Animalia*

Q4. Out of the following characteristics, select the one(s) that may lead to the conclusion of Ctenophora as the outgroup to all other clades of animals. **(10 points)**
(Select the correct option.)

A. Muscles
B. Sensory Organs
C. Hox genes
D. Digestive organs
E. MicroRNA

Q5. Based on Table 1, which of the five phylogenetic trees presented in Figure 7 is the most parsimonious? **(30 points)**
(Select the correct option.)

A. A
B. B
C. C
D. D
E. E

Q6. A group of scientists want to investigate the expression of myogenic regulators in cnidarians and their bilaterian homologues. Which of the following blotting technique(s) should they employ? **(20 points)**

(Select all correct options.)

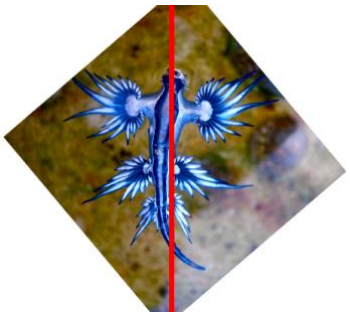
A. Northern Blot
B. Southern Blot
C. Eastern Blot
D. Western Blot

Answers and Explanations

Q1.

Answer: **B, D, C, E, A**

Explanation: We can see that Animal A is clearly bilateral (as seen below) and Animal B is asymmetrical with its odd shape. Animal C has two planes of symmetry. One of the planes of symmetry is obstructed by the view. Animal D has a circular shape with infinite lines of symmetry, as seen in the figure below. Animal E can be divided into five parts when viewing from the top-down.



Q2.

Answer: **FFFT**

Explanation:

A. As stated in the preamble, sponges do not have separate germ layers and any true tissues.
B. Observable in Figure 4, the Ping Pong tree sponge cannot be divided into planes of symmetry passing through its central axis.
C. As stated in the preamble, filter-feeding sponges use this filtration system to capture bacteria and other microorganisms, which are phagocytosed by amoeboid cells. Carnivorous sponges, on the other hand, often have a different feeding mechanism that does not rely on these filters.
D. Most carnivorous sponges have lost their water filtration system due to them no longer using the system for feeding. However, the Ping Pong tree sponge, which has inflated nodules at the end of its branches, uses it to inflate the spheres.

Q3.

Answer: **A**

Explanation: The flagellum (tail-like feature of the organism) is characteristic of choanoflagellates, which is also seen in Cell A.

Q4.

Answer: **E**

Explanation: MicroRNA is the only characteristic that is absent in *Ctenophora* but present in the other clades, suggesting that it is a different group.

	Porifera	*Cnidaria*	*Ctenophora*	*Bilateria*
MicroRNA	**Present**	**Present**	**Absent**	**Present**

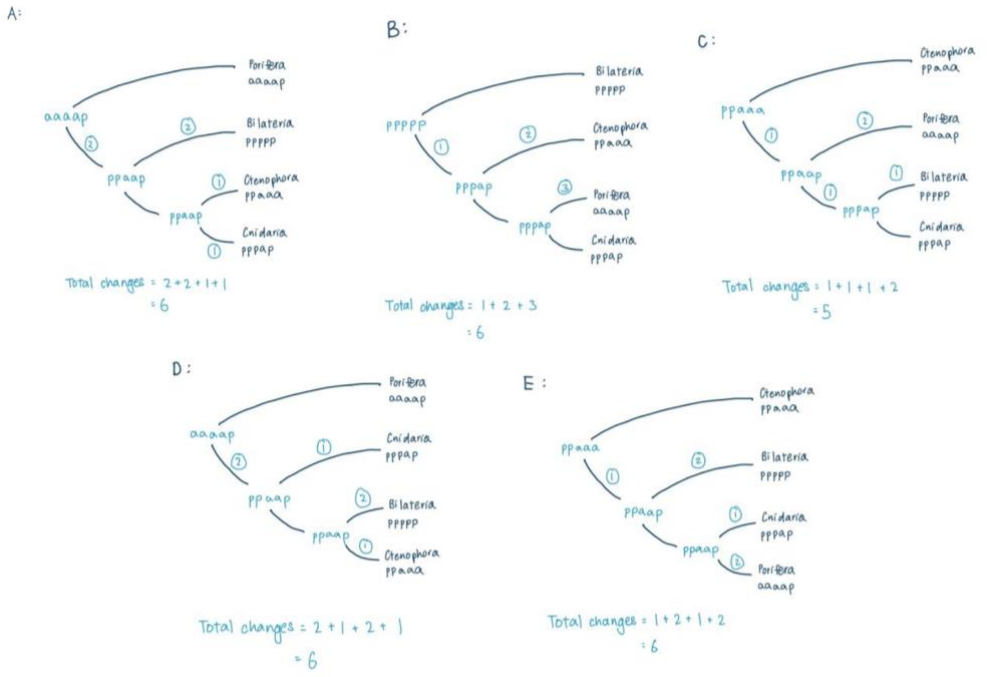
Q5.

Answer: **C**

Explanation: "Most parsimonious" means fewest total evolutionary changes for all traits in each phylogenetic tree.

How to approach the counting:

1. All 5 traits were counted together in each phylogenetic tree (just to minimise efforts).
    a. a denotes Absent, p denotes Present (e.g. aaaap represents traits for *Porifera* in the order of Muscles, Sensory Organs, Hox genes, Digestive organs and MicroRNA respectively).
2. Double counting was avoided (i.e. the situation where a trait was changed to a/p, or vice versa, was only to be changed back to p/a, or vice versa, further down in the tree).
3. The final traits at the end of the tree and the initial traits at the start of the tree were noted down first, and changes to traits were then made along the way to "link" the start and end together.



Since Option C has the least number of total changes out of all the options, it is most parsimonous.

Note: There might be other permutations for traits changes in each tree, but the least number of changes for each tree should still be the same.

Q6.

Answer: **A, D**

Explanation: Expression of genes (and therefore the protein produced) is being investigated. All the cells have DNA and the presence of DNA is not representative of gene expression so we should not be choosing techniques that can analyse the DNA present. Gene expression can be quantified by the mRNA levels as they are transcribed from the genes, as well as the amount of proteins present. Thus, we are looking for techniques that help to analyse the RNA and protein produced.

A. Northern Blot detects RNA presence and hence gene expression.
B. While Southern Blot detects DNA, all cells in general contain DNA and will hence contain the gene regardless of whether the gene is expressed. Hence, we cannot use this technique.
C. This detects post-translational modification in proteins. We are concerned about the expression of proteins. While this technique indirectly detects proteins, we cannot use this technique because it is possible that no post-translational modification occurs and the protein is not detected, which produces a false negative if the protein is actually present.
D. Western Blot detects the presence of the specific proteins.

Credits

Figure 1: GeeksforGeeks. (2022, August 4). Symmetry in animals - definition, types and importance. https://www.geeksforgeeks.org/animal-symmetry/

Figure 2:

Animal A: Rohrlach, S. (2013, 3 March). *Blue dragon-glaucus atlanticus*. Wikipedia. https://commons.wikimedia.org/wiki/File:Blue_dragon-glaucus_atlanticus_(8599051974).jpg

Animal B: Picchetti, G. (2003, 27 June). *Chondrosia reniformis*. Wikipedia. https://commons.wikimedia.org/wiki/File:Chondrosia_reniformis01.jpg

Animal C: Vellutini, B. C. (2013, 23 February). *Comb jelly 2*. Wikipedia. https://commons.wikimedia.org/wiki/File:Comb_jelly_2.jpg

Animal D: Schmahl, G.P. (n.d.). *Coral basics*. Flower Garden Banks National Marine Sanctuary. https://flowergarden.noaa.gov/education/coralbasics.html

Animal E: MDMihaela. (2021, 25 February). *Chondrocladia lyra*. Wikipedia. https://commons.wikimedia.org/wiki/File:Chondrocladia_lyra.jpg

Figure 3: Marine Education Society of Australasia. (n.d.). *Porifera*. http://www.mesa.edu.au/porifera/porifera01.asp

Figure 4: Vacelet, J. (1993, 11 December). *Chondrocladia lampadiglobus*. Wikipedia. https://commons.wikimedia.org/wiki/File:Chondrocladia_lampadiglobus.jpg

Figure 5: Carr, M., Leadbeater, B. S., Hassan, R., Nelson, M., & Baldauf, S. L. (2008). Molecular phylogeny of choanoflagellates, the sister group to metazoa. *Proceedings of the National Academy of Sciences*, *105*(43), 16641–16646. https://doi.org/10.1073/pnas.0801667105

Figure 6: Webster, NS. (2016, 21 April). *Asconoid sponge body plan*. Wikipedia. https://commons.wikimedia.org/wiki/File:Asconoid_Sponge_body_plan.png

Figure 7: Dunn, C. W., Leys, S. P., & Haddock, S. H. D. (2015). The hidden biology of sponges and ctenophores. *Trends in Ecology & Evolution*, *30*(5), 282–291. https://doi.org/10.1016/j.tree.2015.03.003 https://doi.org/10.1016/j.tree.2015.03.003

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Anderson, D. T. (2001). *Invertebrate zoology*. Oxford University Press.

Moroz, L. L., Kocot, K. M., Citarella, M. R., Dosung, S., Norekian, T. P., Povolotskaya, I. S., Grigorenko, A. P., Dailey, C., Berezikov, E., Buckley, K. M., Ptitsyn, A., Reshetov, D., Mukherjee, K., Moroz, T. P., Bobkova, Y., Yu, F., Kapitonov, V. V., Jurka, J., Bobkov, Y. V., … Kohn, A. B. (2014). The ctenophore genome and the evolutionary origins of neural systems. *Nature*, *510*(7503), 109–114. https://doi.org/10.1038/nature13400

VACELET, J. (2006). New Carnivorous Sponges (Porifera, Poecilosclerida) collected from manned submersibles in the Deep Pacific. *Zoological Journal of the Linnean Society*, *148*(4), 553–584. https://doi.org/10.1111/j.1096-3642.2006.00234.x

Ruppert, E. E., Fox, R. S., & Barnes, R. D. (2004). *Invertebrate zoology : a functional evolutionary approach (7th ed.)*. Thomson-Brooks/Cole.

P08: Plants make me spiral

(180 points)

You discovered an unknown organism under the microscope. This organism is an autotroph. Figure 1 shows the unknown organism.

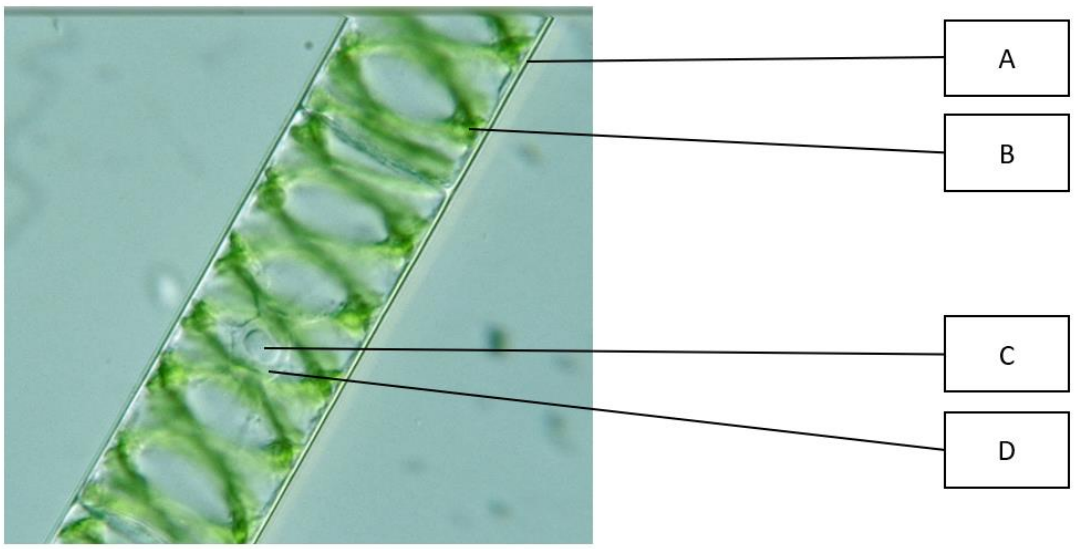


Figure 1: Unknown Organism

Q1. By comparing this organism to a typical plant cell, match the cell structures to their correct descriptions. Not all letters may be used, and each letter may be used more than once. If there is no such cell structure, enter *None*. **(50 points)**
(Match the correct letters to the correct rows.)

Description	**Cell structure (A-D)**
Starch	
Presence of chromatin	
Chlorophyll	
Site of glycolysis	
Contains peptidoglycan	

Small organisms like the organism in Figure 1 can be easily observed under a microscope. There are many different types of microscopes. Figures 2 to 8 show several different images taken by different types of microscopes. The images have been converted to black and white images.

Figure 2: Sporophyte

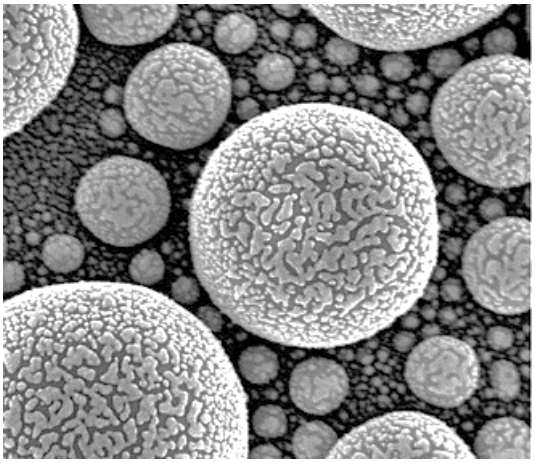


Figure 3: Pollen Grain

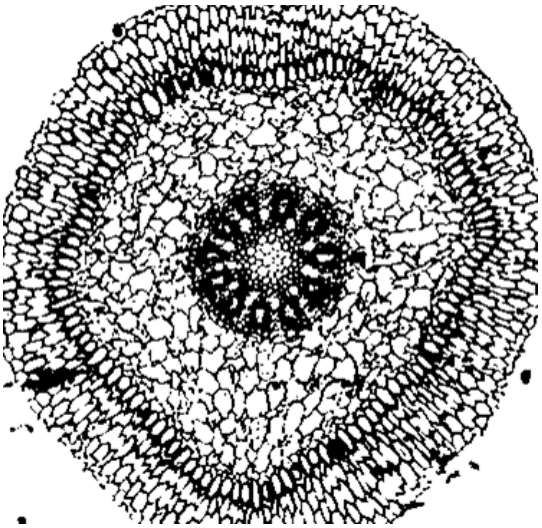


Figure 4: Unknown

Figure 5: Unknown

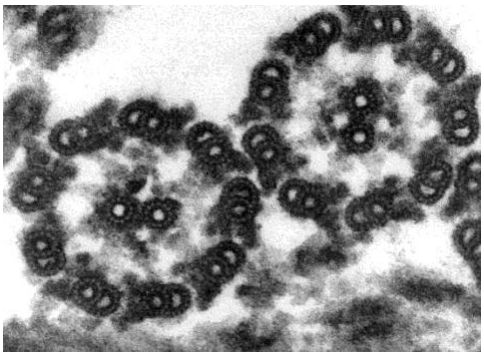


Figure 6: Unknown

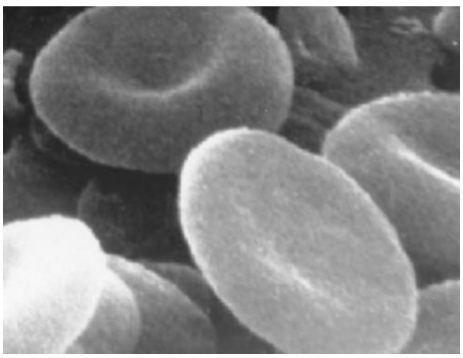


Figure 7: Unknown

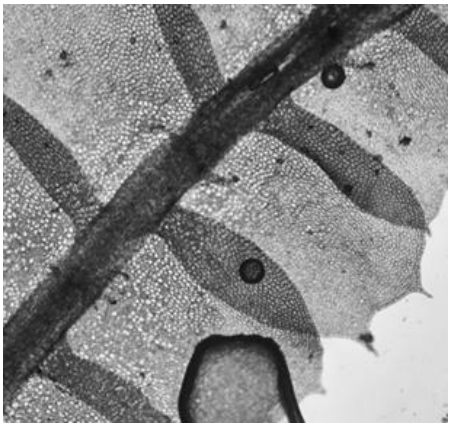


Figure 8: Unknown. Bubbles present due to poor experimental techniques.

Q2. Each of the three figures (Figures 2-4) was captured using one of the four microscopy techniques listed below. Match the microscopy technique used (A-D) to the figures that they were used to capture. Not all techniques may be used, and techniques may be used more than once. **(30 points)**

(Enter the correct letter to each row.)

A. Scanning Electron Microscope
B. Stereo Microscope
C. Light Microscope
D. Transmission Electron Microscope

Figure	**Microscopy technique (A-D)**
2	
3	
4	

Q3. Each of the four figures (Figures 5-8) was captured using one of the four microscopy techniques listed below. Match the microscopy technique used (A-D) to the figures that they were used to capture. Not all techniques may be used, and techniques may be used more than once. **(40 points)**

(Enter the correct letter to each row.)

A. Scanning Electron Microscope
B. Stereo Microscope
C. Light Microscope
D. Transmission Electron Microscope

Figure	**Microscopy technique (A-D)**
5	
6	
7	
8	

Q4. Which statement is true regarding the use of a light microscope? **(20 points)**
(Select the correct option.)

A. Oil needs to be used as a medium between the objective lens and the glass slide.
B. The condenser increases the magnification of the image.
C. The magnification of the image is calculated by the sum of the magnification of ocular lens and the objective lens.
D. The nosepiece is used to change the magnification of the image.
E. Viewing the microscope image with one eye results in a 2D image but viewing it with both eyes results in a 3D image.

Q5. You are examining an onion cell under a microscope and used the microscope to take a picture of the cell. The magnification of the image is 500x. You measured the image and found that the length of the image of the cell is 15.4cm, and the width of the image of the cell is 7.2cm. Assuming that the cell is a perfect rectangle, calculate the actual area in μm^2 occupied by the cell. **(20 points)**
(Enter a number correct to 3 s.f. Do not leave any units.)

Answers and Explanations

Q1.

Answer: **B, D, B, C, None**

Explanation: Figure 1 shows a spirogyra. From Part A to D respectively, the cell structures are: Cell wall, chloroplast, and nucleus.

Analysing each row:

A. Starch is stored in the chloroplasts and amyloplasts.
B. The chromatin is found in the nucleus.
C. Chlorophyll is found in the chloroplasts.
D. The site of glycolysis is in the cytosol not the mitochondria.
E. Peptidoglycan cell walls are found in bacterial cells. There is no peptidoglycan cell wall here.

Q2 and Q3.

Answer: **Q2: BAC; Q3: BDAC**

Explanation: Both stereo microscopes and scanning electron microscopes (SEM) produce 3D images, while the other two produce 2D images. Stereo microscopy is used to view larger objects, while SEM and transmission electron microscopy (TEM) is used to view smaller objects.

Analysing each figure:

2. Figure 2 is an image of a moss sporophyte. The image is 3D, and the sporophyte is a large part of the plant. Moreover, we can see that the image contains tissues rather than individual cells, so this is an image captured using a stereo microscope.
3. Figure 3 is an image of pollen grains which are usually of sizes in the micrometers. The image is also 3D so it must have been captured by an SEM.
4. Figure 4 is clearly taken using a light microscope as it shows the individual cells of a cross section of an orchid root.
5. Figure 5 is taken with a stereo microscope as it is 3D and shows the underside of a fern which are relatively large.
6. Figure 6 clearly shows the 9+2 structure of microtubules which are very small as they are found inside cells. This cannot be taken with a light microscope as they would be too small to be visible. As this image is 2D, it must be taken with a TEM.

7. Figure 7 is clearly a 3D image of a red blood cell which would mean it was taken with SEM and not a stereo microscope as red blood cells are very small.
8. Figure 8 shows the underside of a liverwort, showing individual cells. As it is not a 3D image, it must be taken with a light microscope. The poor experimental techniques causing bubbles to appear on the image also implies that the microscopy technique is light microscopy as only with it will there be bubbles present due to bubbles between the cover slip and the specimen.

Q4.

Answer: **D**

Explanation:

A. Oil only needs to be used if the objective lens requires it.
B. The condenser focusses the light on the specimen. The objective lenses and eyepieces increase the magnification of the image.
C. The magnification of the image is calculated by the **product** of the magnification of ocular lens and the objective lens, not the sum.
D. The nosepiece can be rotated to change the objective lens used to alter the magnification of the image.
E. Either way there will only be a 2D image as there is only one light source.

Q5.

Answer: **44400**

Explanation: One must be incredibly careful during conversions because $1m^2$ is not equal to $1 \times 10^6 mm^2$ but $1 \times 10^{12} mm^2$ as the micrometer prefix is applied to the square term, so it must be evaluated twice.

First, convert the lengths in terms of mm: Length = $154000mm$ and Width = $72000mm$. The actual lengths can be found by dividing by the magnification of 500: Length = $308mm$ and Width = $144mm$. Thus, the area is $308 \times 144 = 44400mm^2$. This method is ideal because we only multiply to find the area at the end, so we eliminate the need for any repeated counting of magnifications or prefixes.

Here are two alternative methods to find the area:

$$Area = \frac{154000 \times 72000}{500 \times 500}$$

This method finds the area first then divides by the magnification twice as the magnification is applied to both length and width.

$$Area = \frac{15.4 \times 7.2 \times 10^4 \times 10^4}{500 \times 500}$$

This method uses the values in cm and then multiplies them by 10^4 twice as it is applied to the length and the width.

P09: Romance of the Three Kingdoms

(100 points)

In a country conjured from dreams, there exist kingdoms A, B and C. Each kingdom consists of an infinitely large population of spiders. Our study only focuses on gene X, which has three alleles: P, Q and R. Allele P is fixed in the population of kingdom A spiders, Q is fixed for those in B and R for those in C.

Gene X codes for the number of eyes the spiders have. All spiders from kingdom A have 3 eyes, all spiders from B have 4, and all spiders living in C have 2. We pick some spiders and have them mate with one another to get the following results. All spiders who were born from a cross between spiders of kingdoms:

- A and B have 4 eyes.
- A and C have 3 eyes.

Q1. What is the dominance hierarchy of the three alleles? Assume complete dominance. A *greater than* sign (>) indicates dominance (e.g. X > Y means allele X is dominant while allele Y is recessive) and an *equal sign* (=) indicates equal dominance. **(10 points)**
(Select the correct option.)

A. P > Q > R
B. P > R > Q
C. Q > P > R
D. Q > R > P
E. R > P > Q
F. R > Q > P
G. P = Q > R
H. Q = R > P
I. R = P > Q
J. P = Q = R

Q2. How many eyes would a spider with parents from kingdoms B and C have? **(10 points)**
(Enter a whole number.)

We now introduce equal proportions of spiders from the three kingdoms into a new country (the P generation) and they undergo random mating. However, a disease in this country kills any spider with 4 eyes as soon as they are conceived. This does not affect the parent spiders originally from kingdom B but affects all future offspring they have (i.e., only the F1 generation is affected).

Q3. What proportion of all spiders survive past hatching in the F1 generation? **(20 points)**
(Enter your answer as a percentage to the nearest whole number. Do not include the percent (%) sign.)

Q4. What will the frequencies of the three alleles be after a long time in this new country? **(30 points)**
(*Enter the correct answer as a decimal correct to 3 s.f. to each row.)*

Allele	**Frequency**
P	
Q	
R	

Q5. Let us consider the country at a time when all alleles have reached new equilibrium values, as hinted in **Q4**. At this point in time, what is the probability that the offspring of two spiders, each with 3 eyes, has 2 eyes? **(30 points)**
(Enter your answer as a decimal correct to 3 s.f.)

Answers and Explanations

Q1.

Answer: **C**

Explanation: Kingdom A spiders have the genotype PP, kingdom B spiders have genotype QQ and kingdom C spiders have genotype RR. Crossing spiders from kingdoms A and B result in offspring, all with genotype PQ, while the cross between kingdoms A and C result in offspring with only the genotype PR. If we arrange these genotypes in order of the number of eyes the spiders have:

- QQ – 4
- PQ – 4, or QP – 4
- PP – 3
- PR – 3
- RR – 2

From the first two, we can deduce Q is dominant over P and from the third and fourth, we can deduce P is dominant over R. This implies Q must also be dominant over R.

Q2.

Answer: **4**

Explanation: These offspring would all have genotype QR. As Q is dominant over R, they would have 4 eyes.

Q3.

Answer: **44**

Explanation: Let us represent the frequencies of alleles P, Q and R as p, q and r respectively. We are told equal proportions of the three kingdoms' spiders are combined to form a fourth population, so we can reasonably say $p_i = q_i = r_i = \frac{1}{3}$ (as $p + q + r = 1$).

Just like the genotypic form of the Hardy-Weinberg equation for two alleles, we can derive one more for three alleles: $(p + q + r)^2 = 1^2 \Rightarrow p^2 + q^2 + r^2 + 2pq + 2pr + 2qr = 1$. If all offspring with 4 eyes are killed, the terms q^2, pq and qr become zero instantly. The proportion of offspring that survive is hence $p^2 + r^2 + 2pr = \left(\frac{1}{3}\right)^2 + \left(\frac{1}{3}\right)^2 + 2\left(\frac{1}{3}\right)\left(\frac{1}{3}\right) = \frac{4}{9}$ which is approximately 44%.

Q4.

Answer: **0.5, 0, 0.5**

Explanation: You can instantly see any spiders with allele Q (QQ, QP or QR) die in the F1 generation. This implies immediately from F1 onwards, $q = 0$. By symmetry, p and r will then match one another to result in equal proportions of 0.5 each.

Q5.

Answer: **0.111**

Explanation: This question can be thought of as a simpler mathematical one testing knowledge of probability. We know that $p = r = 0.5$, so the 3-eye spiders can either be PP or PR. 2-eye spiders can only have the genotype RR so that means both parents need to be PR. The probability of picking a PR-parent from a population consisting of PP and PR is $\frac{2pr}{p^2 + 2pr}$. As two parents need to be picked, and the probability of getting an RR-offspring is $\frac{1}{4}$ (50% chance of getting the R allele from either parent), the final ratio becomes $\frac{1}{4} \times \left[\frac{2pr}{p^2 + 2pr}\right]^2 = \frac{1}{4} \times \left[\frac{0.5}{0.25 + 0.5}\right]^2 = \frac{1}{9}$, which is approximately 0.111.

P10: This is a BLAST

(190 points)

The 2003 Nobel Prize in Chemistry was awarded to Dr Peter Agre for his discovery of aquaporin proteins. Aquaporins are a family of membrane proteins that facilitate the transport of water across cell membranes and hence help maintain water homeostasis in cells. Water molecules are able to move across cell membranes via simple diffusion or facilitated diffusion via these aquaporin channels.

Mutations in the aquaporin (AQP) genes that code for the aquaporins can lead to different diseases. You are provided with the mutated sequence of aquaporin protein X from an individual with mutations in one of the aquaporin proteins. As there are many different aquaporin proteins, you need to make use of the Basic Local Alignment Search Tool (BLAST) to determine which aquaporin protein Protein Sequence X belongs to.

BLAST is a tool that finds regions of similarity between biological sequences by comparing sequences of nucleotides or proteins to known sequence databases to find the closest matches. You will first be making use of the BLAST tool to determine which aquaporin protein the unknown protein belongs to.

Procedure

1. In a web browser, load *https://blast.ncbi.nlm.nih.gov/*.
2. Select the most appropriate BLAST option in the Web BLAST section. You need to select the one that allows you to input your protein sequence and subsequently outputs the most similar protein sequences in the databases.
3. Enter the mutated sequence of protein X into the query box.
4. Under Databases, select “Standard databases (nr etc.):”.
5. Under Standard, select “Non-redundant protein sequences (nr)”. Leave the optional selections blank.
6. Under Algorithm parameters, leave everything else as default.
7. Click the “BLAST” button.

You will be redirected to a Format Request Page as BLAST compares your protein sequence against their databases. Once the job request is complete, you will be redirected to the results page.

The E-value refers to the number of expected hits of similar quality (score) that could be found just by chance alone. Hence, the lower the E-value, the better the alignment.

8. Select the protein sequence with the best E-value and highest percent identity score (Per. Ident). Ensure that the protein chosen is found in humans.

Q1. What is the identity of Aquaporin protein X? **(20 points)**
(Select the correct option.)

A. Aquaporin-1
B. Aquaporin-2
C. Aquaporin-3
D. Aquaporin-4
E. Aquaporin-5
F. Aquaporin-6
G. Aquaporin-7
H. Aquaporin-8
I. Aquaporin-9
J. Aquaporin-10
K. Aquaporin-11

Amino acids can be classified based on their physical and chemical properties. Figure 1 shows a Venn diagram of the amino acids and their properties.

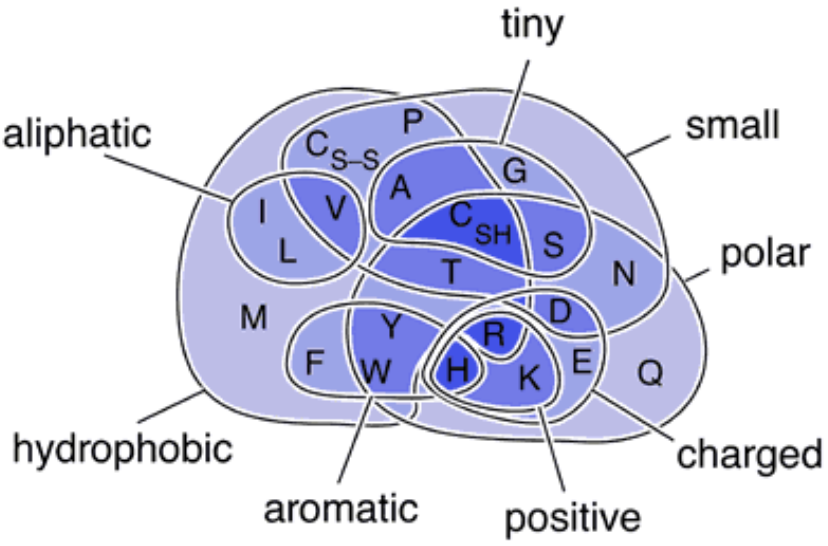


Figure 1: Amino Acid Properties.

9. Select the alignment view “Pairwise with dots for identities”.

Q2. How many amino acid mutations are there in the mutated sequence? **(10 points)**
(Enter a whole number.)

The interface will show you two rows: Query, and Sbjct (Subject). The former is the sequence which you uploaded, while the latter is the sequence matched from the database. The dots represent that the amino acid is the same at that position, while the red amino acids represent a mismatch in amino acid at that position.

Q3. At which position(s) is there an amino acid mutation from polar to hydrophobic? If there are more than one positions, enter your answer as the **sum** of all the positions. **(20 points)**
(Enter a whole number.)

Q4. At which position(s) is there an amino acid mutation from an amino acid with a charge to an amino acid with a **different** charge? If there are more than one positions, enter your answer as the **sum** of all the positions. **(20 points)**
(Enter a whole number.)

We would also like to see the 3D structure of the normal aquaporin protein X in humans and how variants affect it using the Universal Protein Resource (UniProt).

10. Load *https://www.uniprot.org/* on a web browser.
11. Search for the protein using the name of the protein in **Q1**.
12. Select the entry that is your protein. Ensure you chose the correct species, and that the entry is a reviewed entry by Swiss-Prot (indicated by a yellow file icon with a star).
13. Select "Variant Viewer".

Variants in genes can be classified into one of five clinical significances: Pathogenic, Likely pathogenic, Variant of Uncertain Significance (VUS), Likely benign, and Benign.

Q5. What is the clinical significance of the third last mutation in the unknown protein sequence? **(20 points)**
(Select the correct option.)

A. Pathogenic
B. Likely pathogenic
C. VUS
D. Likely benign
E. Benign
F. Not reported

14. Select "Entry".
15. Scroll down to "Sequence".
16. Store the normal sequence of protein X in a .txt file.

Q6. With reference solely to the information on UniProt regarding this entry, indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. There are many alpha helices in the secondary structure of Protein X.
B. The gene coding for Protein X can be found on chromosome 9.
C. Protein X is likely transported to the smooth endoplasmic reticulum and the Golgi apparatus for addition of a carbohydrate chain during post-translational modification.
D. A loss-of-function mutation of Protein X will result in a slower rate of uptake of water by cells in the small intestine.

Next, we will use the Protein Parameters (ProtParam) tool to predict the changes in the physical and chemical parameters of mutated protein X as compared to the normal protein X.

17. Load *https://web.expasy.org/protparam/*.
18. Copy and paste the normal sequence of Protein X into the query box.
19. Press the "Compute parameters" button.
20. Repeat steps 18 and 19 for the mutated protein sequence.

The isoelectric point (pI) of an amino acid is the pH at which it bears no net charge. For an amino acid with no polar side chain, it has a net charge of zero at physiological pH as it is in its zwitterionic form.

Q7. What is the theoretical pI of normal protein X and the mutated protein X? **(20 points)**
(Enter your answer correct to 3 s.f. to each row.)

Protein	**Theoretical pI**
Normal Protein X	
Mutated Protein X	

Other than the mutated protein sequence X, you are also provided with four other mutated sequences of different aquaporin proteins. You are tasked to find the evolutionary relationship between the five aquaporin protein sequences. To do so, we will make use of a multiple sequence alignment programme called ClustalW.

21. Load *https://www.genome.jp/tools-bin/clustalw*.
22. Copy and paste Protein Sequences A to D into the query box. Do not edit anything.
23. You will also need to include the **normal** sequence of Protein X in the search query.

Protein sequences entered in the search query must be in the FASTA format. The FASTA format comprises of the header and the sequence. The header starts with a "more than" sign (>), followed by the name of the sequence without spaces. A carriage return is inserted or the "enter" key is clicked to continue on the next line, and the protein sequence is inserted. As an example, the FASTA format has already been done for you for Unknown proteins A to D.

24. Format the **normal** protein sequence in **Q1** in the FASTA format and paste it at the bottom of the search query.
25. Select "CLUSTAL" as the output format.
26. Select "FAST/APPROXIMATE" for Pairwise Alignment.
27. Leave the "more Detail Parameters" as default.
28. Click the "Execute Multiple Alignment" button.
29. Click the dropdown menu and select "FastTree".
30. Click the "Exec" button.
31. Once the job is completed, click "without branch length".

You should obtain a phylogram similar to that in Figure 2.

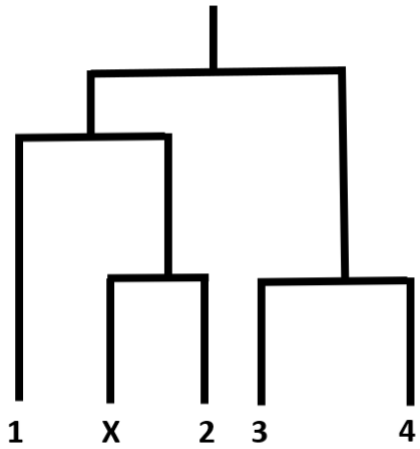


Figure 2: Phylogram. X represents the **normal** protein sequence X.

Q8. Match the unknown protein sequences A-D to the correct numbers in Figure 2. Note that the letter corresponding to number 3 in Figure 2 comes **alphabetically before** that of number 4. **(40 points)**

(Enter the correct letter to the correct row.)

Number in Figure 1	**Protein Sequence (A-D)**
1	
2	
3	
4	

P10 – Resources

Links in the problem can be found here:

- BLAST: https://blast.ncbi.nlm.nih.gov/
- UniProt: https://www.uniprot.org/
- ProtParam: https://web.expasy.org/protparam/
- ClustalW: https://www.genome.jp/tools-bin/clustalw

>MutatedProteinSequenceX

MWELRSIAFSRAVFAEFLATLLFVFFGLGSALNWPQALPSVLQIPMAPGLGIGTLVQALGHISGAHI
NPAVTVACLVGCHVSVLRAAFYVAAMLLGAVAGAALLHEITPADIRGDLAVNALSNSTTAGQAVT
VELFLTLQLVLCIFASTDERRGENPGTPALSIGFSVALGDLLGIHYTGCSMNPARSLTPAVVTGKFDD
HWVFWIGPVVGAILGSLLYNYVLAPPAKSLSERLAVLKNLEPDTDWEEREVRRRQSVELHSPQSLP
RGTKA

>UnknownProteinSequenceA

MKKEVCSVAFLKAVFAEFLATLIFVFFGLGSALKWTFALPTILQIALAFGLAIGTLAQALGPVSGGHI
NPAITLALLVGNQISLLRAFFYVAAALVGAIAGAGILYGVAPLNARGNLAVNALNNNTTQGQAMV
VELILTFQLALCIFASTDSRRTSPVGSPALSIGLSVTLGHLVGIYHTGCSMNPARSFGPAVVMNRFSP
AVLVFWVGPIVGAVLAAILYFYLLFPNSLSLSERVAIIKGTYEPDEDWEEQNEERKKTMELTTR

>UnknownProteinSequenceB

MSGEIAMCEPEFGNDKAREPSVGGRWRLMWYERFVQPCLVELLGSALFIFIGCLSVIENGTDTGLL
QPALAHGLALGLVIATLGNPSGGHFNPAVSLAAMLIGGLNLVMLLPYWVSFLLGGMLGAALAKA
VSFWERFWNASGAAFATVQEQGQVAGALVAEIILTTLLALAVCMGAINEKTKGPLAPFSIGFAVTV
DILAGGPVSGGCMNPARAFGPAVVRNHWNFHWISTAGPLLAGLLVGLLIRCFIGDGKTRLILKAR

>UnknownProteinSequenceC

MVFTQAPAEIMGHLRIRSLLARQCLAESLGVFVLMLLTQGAVAQAVTSGETKGNFFTMFLAGSLA
VTIAIYVGGNVSGAHLNPAFSLAMAIVGRLVVVKLPIYILVQLLSAFCASDATYVLYHDALQNYTG
MNLTVTGPKETASIFGTYPAPYLSLNNGFLDQVLGTAMLIVGLLAILDRRNKGVPAGLEPVVVGML
ILALGLSMGANCGIPLNPARDLGPRLFTYVAGWGPEVFSAGNGWWWVPVVAPLVGAYVGTATYQ
LLVALHHPEGPEPAQDLVSAQHKPSELETPASAQMLECKL

>UnknownProteinSequenceD

MVQASGHRRSTRGSKMVSWSVIAKIQEILQRKMVREFLAEFMSTYVMMVFGLGSVAHMVLNKKY
GSYLGVNLGFGFGVAMGRHVAGRISGAHMNAAVTFANCALGRVPWRKFPVYVLGQFLNMFLAA
ATIYSLFYTAILHFSGSQLMVTGPVATAGIFATYLPDHMTSWRGFLNEAWLTGMLQLCLFAITDQE
NNPALPGTEALVIGILVPIIGVSLGMNTGYAINPSRDLPKRIFTFIAGWGKQVFSNGENWWWVPVVA
PLLGAYLGGIIYLVFIGSTIPREPLKLEDSVASEDHGITVLPKMGSHEPTISPLTPVSVSPANRSSVHPA
PPLHESMALEHF

Answers and Explanations

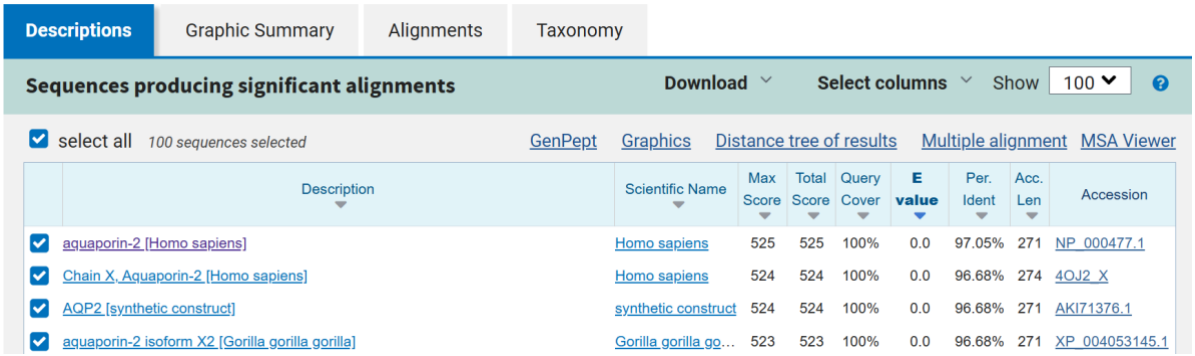
This problem is rather trivial and introduces participants to the exciting world of bioinformatics. However, it is important to follow the instructions carefully to avoid any mistakes. All links and information are accurate at the time of the contest.

Participants were provided with the links in the problem as well as the required protein sequences during the contest.

Q1.

Answer: **B**

Explanation: Protein BLAST (blastp) should be selected as we are passing a protein sequence and hoping to find a similar protein sequence. After Step 7, you should get this following result. It may take a few seconds to a few minutes for the job request to process.



From: https://blast.ncbi.nlm.nih.gov/Blast.cgi

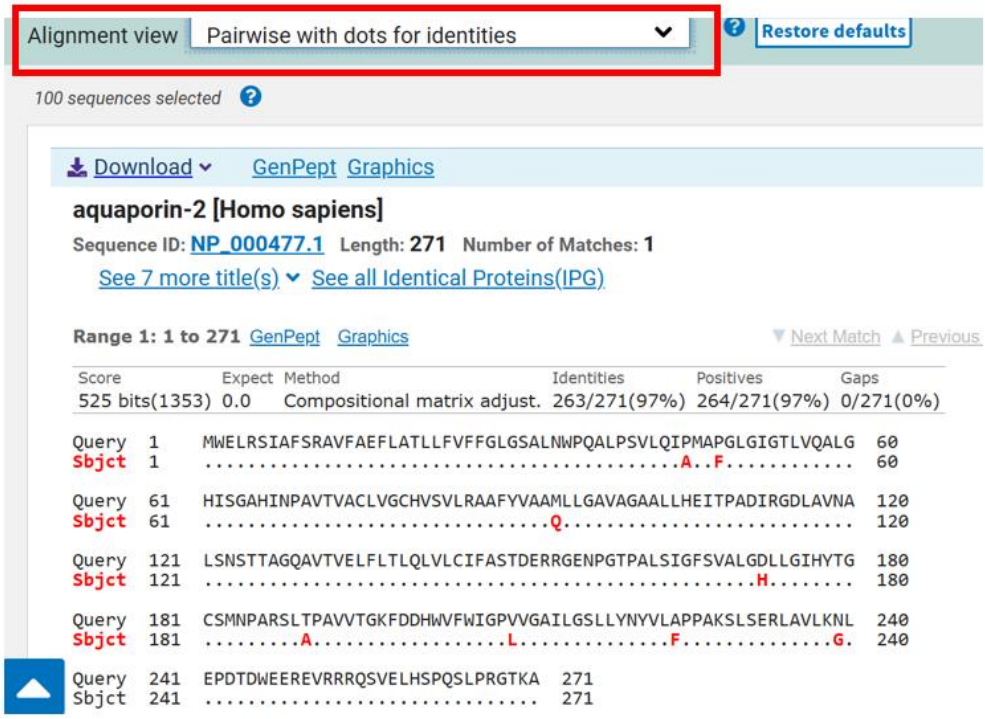
We select the first entry i.e. aquaporin-2 [Homo sapiens] as it has the lowest E-value and the highest percent identity. This aquaporin protein is also found in humans which are *Homo sapiens*.

Hence, the identity of the aquaporin protein is Aquaporin-2.

Q2.

Answer: **8**

Explanation: After selecting “Pairwise with dots for identities”, you should see this result:



From: https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_NP_000477

Each letter in red represents a mismatch in the amino acid between the normal sequence and the mutated query sequence. Hence, we can count the number of mutated amino acids which is 8.

For reference, here is the mutated sequence and normal sequence respectively.

Mutated sequence

MWELRSIAFSRAVFAEFLATLLFVFFGLGSALNWPQALPSVLQIPMAPGLGIGTLVQALGHISGAHI
NPAVTVACLVGCHVSVLRAAFYVAAMLLGAVAGAALLHEITPADIRGDLAVNALSNSTTAGQAVT
VELFLTLQLVLCIFASTDERRGENPGTPALSIGFSVALGDLLGIHYTGCSMNPARSLTPAVVTGKFDD
HWVFWIGPVVGAILGSLLYNYVLAPPAKSLSERLAVLKNLEPDTDWEEREVRRRQSVELHSPQSLP
RGTKA

Normal sequence

MWELRSIAFSRAVFAEFLATLLFVFFGLGSALNWPQALPSVLQIAMAFGLGIGTLVQALGHISGAHI
NPAVTVACLVGCHVSVLRAAFYVAAQLLGAVAGAALLHEITPADIRGDLAVNALSNSTTAGQAVT
VELFLTLQLVLCIFASTDERRGENPGTPALSIGFSVALGHLLGIHYTGCSMNPARSLAPAVVTGKFD
DHWVFWIGPLVGAILGSLLYNYVLFPPAKSLSERLAVLKGLEPDTDWEEREVRRRQSVELHSPQSL
PRGTKA

Q3.

Answer: **93**

Explanation: Using the Venn Diagram provided in Figure 1, we can see that the **mutation of Q to M** at position 92 is the only change from a polar to hydrophobic amino acid. Do note that the red amino acid is the subject and is hence the **normal sequence**.

Q4.

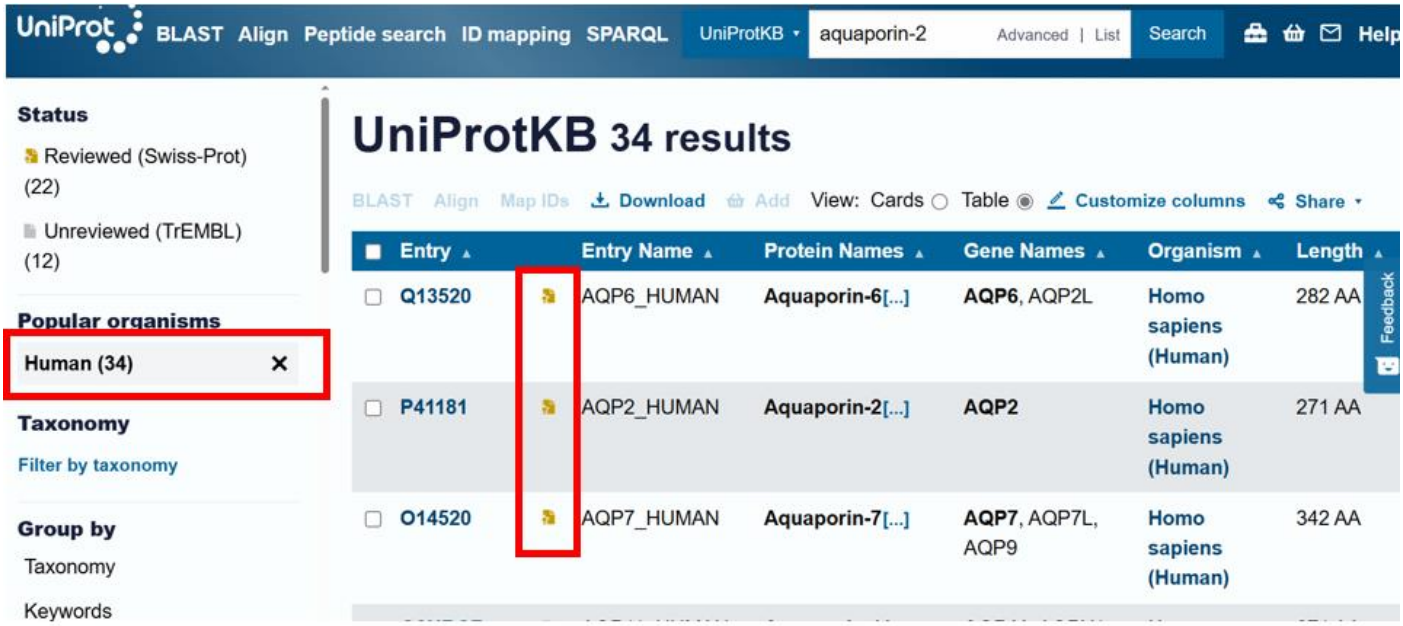
Answer: **172**

Explanation: Using the Venn Diagram provided in Figure 1, we can see that the **mutation of H to D** at position 172 is the only mutation which caused a change in charge.

Q5.

Answer: **D**

Explanation: We search in UniProt the protein Aquaporin-2. We select human in the filter and select the only entry of aquaporin-2 which is Swiss-Prot reviewed, which is entry **P41181**.



From: https://www.uniprot.org/uniprotkb?query=aquaporin-2&facets=model_organism%3A9606

We then click “Variant Viewer”. The third last mutation is that at position 209 which is a mutation from L>V. We scroll down to variant 209 and observe that such a mutation is classified as likely benign, which is our answer.

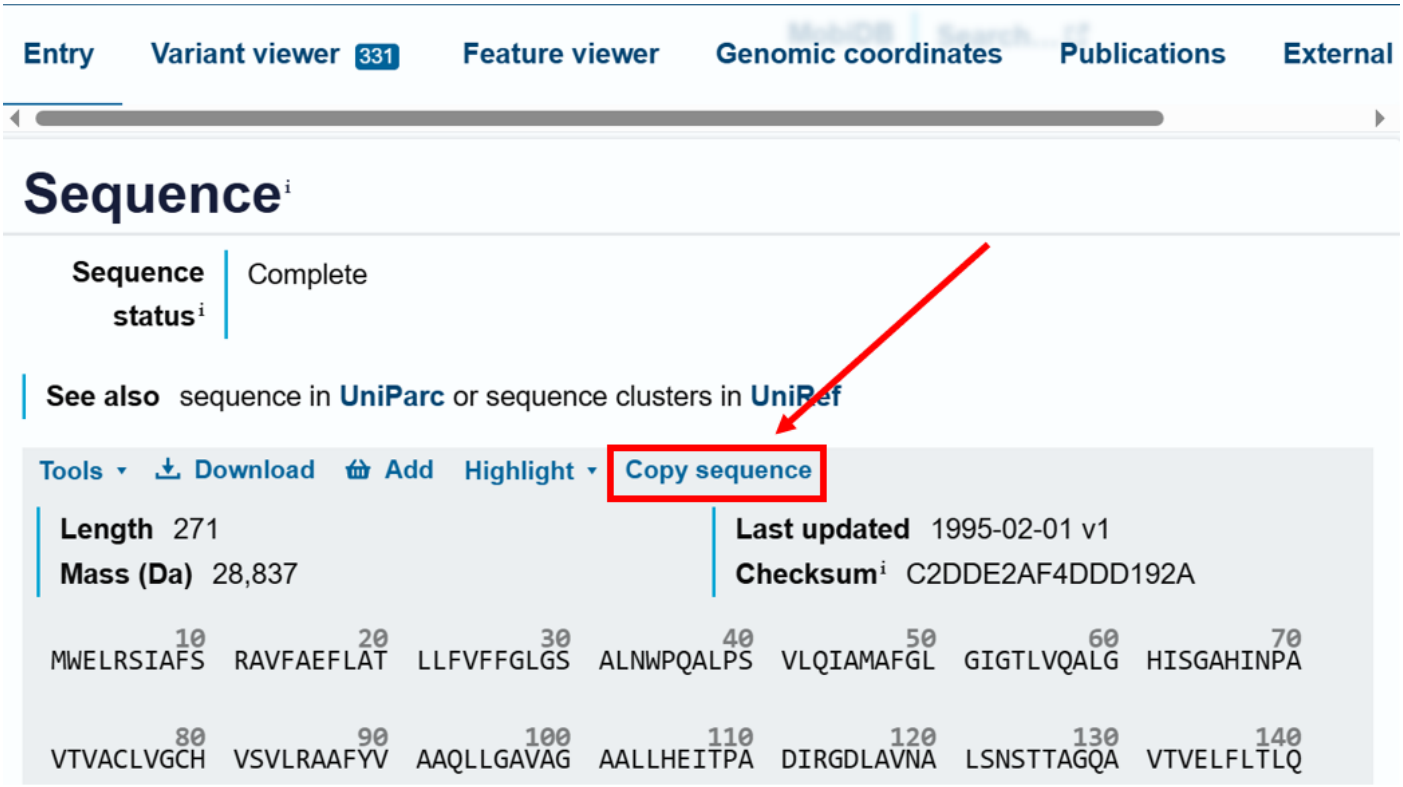
▶ rs1163056099	208	P>T			TOPMed gnomAD
▶ RCV001109687 rs889586478	209	L>P	Diabetes insipidus, nephrogenic, autosomal (ClinVar)	Variant of uncertain significance (Ensembl, ClinVar)	ClinVar TOPMed dbSNP gnomAD
▶ RCV002207002 rs1555165381	209	L>V		Likely benign (Ensembl, ClinVar)	ClinVar TOPMed dbSNP
▶ rs752925390	210	V>M			ExAC gnomAD

From: https://www.uniprot.org/uniprotkb/P41181/variant-viewer

Q6.

Answer: **TFFF**

Explanation: We are told to store the normal sequence of aquaporin-2 on a notepad.



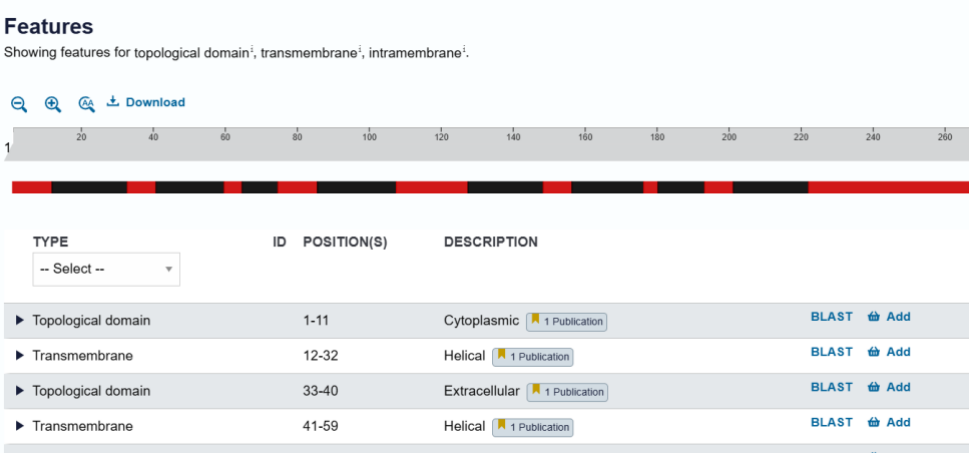
From: https://www.uniprot.org/uniprotkb/P41181/entry

We can click “Copy sequence” and paste it in a .txt file to save it.

Now we need to observe the information on the page to solve several statements. The following screenshots are all taken from https://www.uniprot.org/uniprotkb/P41181/entry.

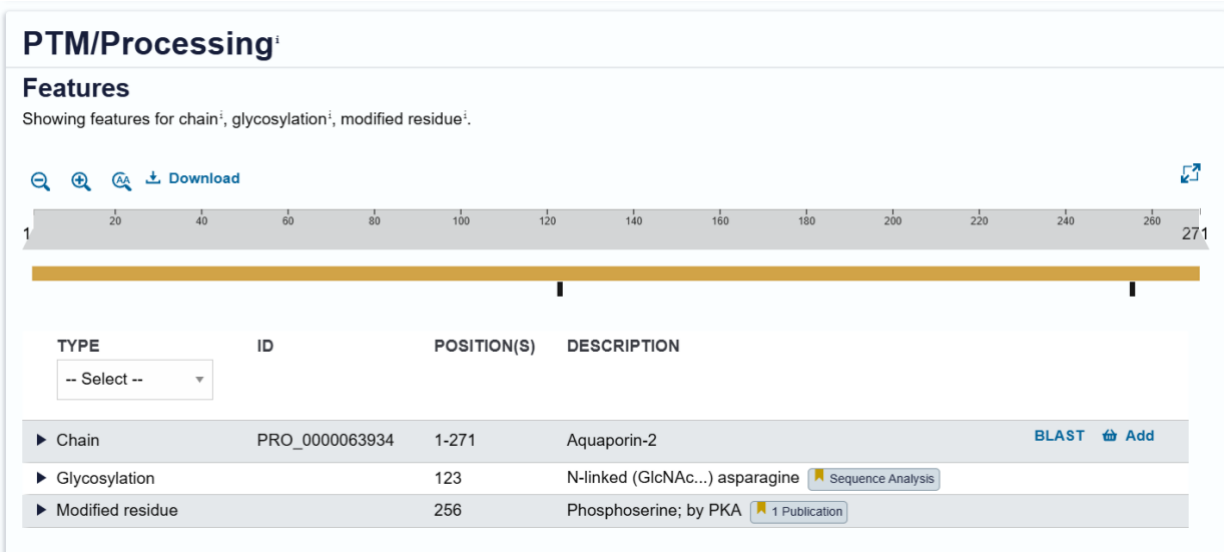
A. We can observe the 3D structure of aquaporin-2 and see that there are many alpha-helices.

This is corroborated by the features tab which shows the regions with helices.



B. Under Proteomes, we can see that the gene is actually found on chromosome 12.

C. While it is true that aquaporin-2 undergoes glycosylation as seen below, the statement is false because glycosylation occurs in the rough endoplasmic reticulum and Golgi apparatus and not the smooth endoplasmic reticulum.



PTM/Processing

Features

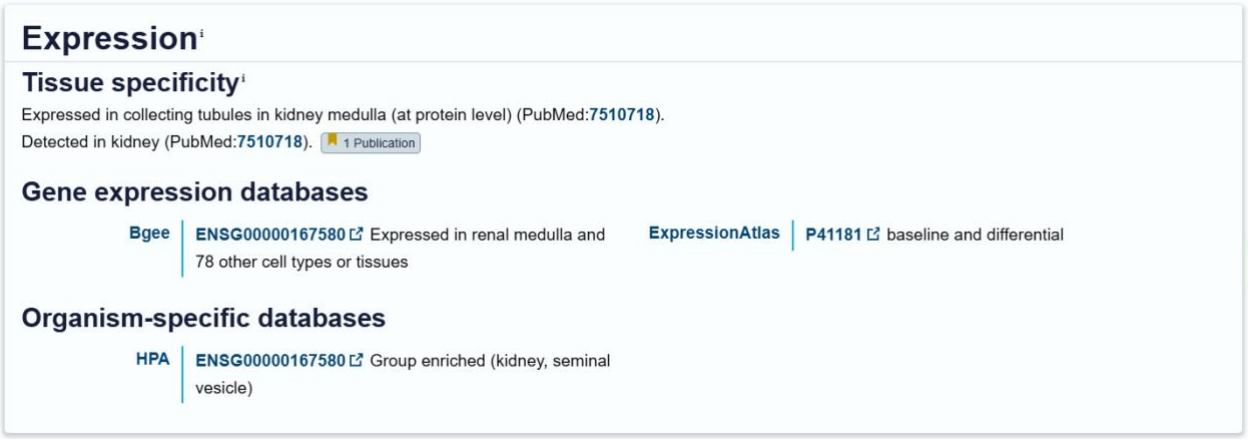
Showing features for chain, glycosylation, modified residue.

Download

1 20 40 60 80 100 120 140 160 180 200 220 240 260 271

TYPE	ID	POSITION(S)	DESCRIPTION	
-- Select --				
Chain	PRO_0000063934	1-271	Aquaporin-2	BLAST Add
Glycosylation		123	N-linked (GlcNAc...) asparagine Sequence Analysis	
Modified residue		256	Phosphoserine; by PKA 1 Publication	

D. It is stated that aquaporin-2 is specific to the kidneys and thus any mutations to aquaporin-2 will not directly affect the absorption of water in the small intestines.



Expression

Tissue specificity

Expressed in collecting tubules in kidney medulla (at protein level) (PubMed:7510718).
Detected in kidney (PubMed:7510718). 1 Publication

Gene expression databases

Bgee | ENSG00000167580 Expressed in renal medulla and 78 other cell types or tissues

ExpressionAtlas | P41181 baseline and differential

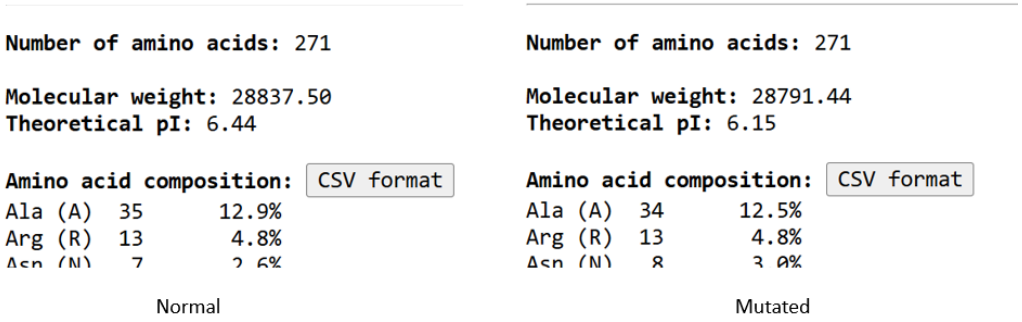
Organism-specific databases

HPA | ENSG00000167580 Group enriched (kidney, seminal vesicle)

Q7.

Answer: **6.44, 6.15**

Explanation: We enter the amino acid sequence of the normal protein and mutated protein to obtain the following results:



Normal Mutated

From: https://web.expasy.org/cgi-bin/protparam/protparam

Q8.

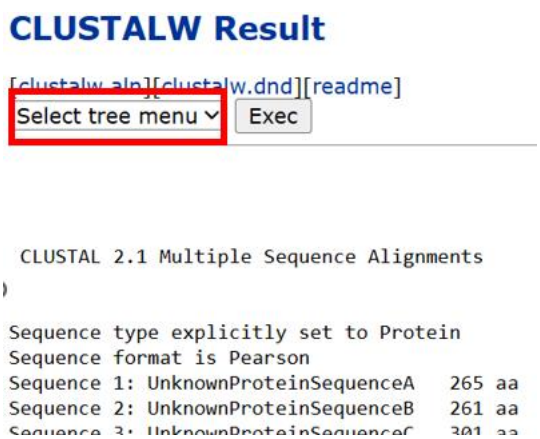
Answer: **B, A, C, D**

Explanation: The formatted FASTA sequence of the normal protein should look like this:

>NormalProteinX
MWELRSIAFSRAVFAEFLATLLFVFFGLGSALNWPQALPSVLQIAMAFGLGIGTLVQALGHISGAHI
NPAVTVACLVGCHVSVLRAAFYVAAQLLGAVAGAALLHEITPADIRGDLAVNALSNSTTAGQAVT
VELFLTLQLVLCIFASTDERRGENPGTPALSIGFSVALGHLLGIHYTGCSMNPARSLAPAVVTGKFD
DHWVFWIGPLVGAILGSLLYNYVLFPPAKSLSERLAVLKGLEPDTDWEEREVRRRQSVELHSPQSL
PRGTKA

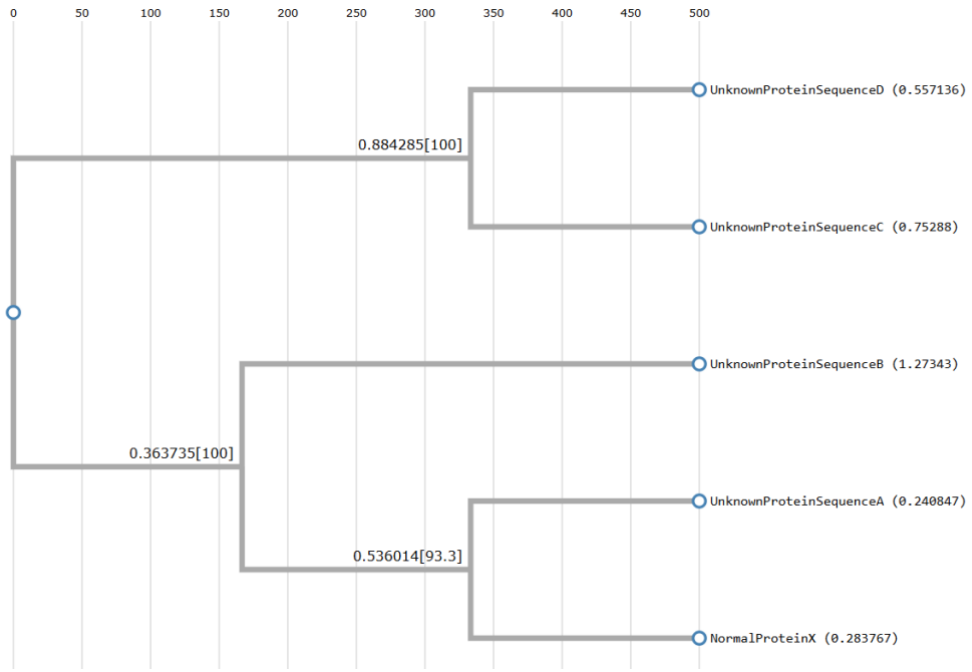
You may name the protein however you prefer.

After pressing the “Execute Multiple Alignment” button, we get this:



From: https://www.genome.jp/tools-bin/clustalw

After selecting FastTree, the job will take a while to process. After the job is completed, you should see a tree similar to the one below. The branch length labels may be different.



From: https://www.genome.jp/tools-bin/ete?id=24071418571140225c85517a25b0dcbce06c097a459f3571d247

Comparing this tree to that in Figure 2, we can match the numbers to the letters. Note that while the tree may look slightly different, they are actually equivalent as you can rotate the clades about each node.

Since the letter corresponding to number 3 comes alphabetically before that of number 4, number 3 is Protein C and number 4 is Protein D.

P11: Hold Dear to Me

(150 points)

Parabiosis is a technique that involves the surgical joining of two living organisms such that they develop a single, shared physiological system as their blood vessels are connected together. This allows blood and its contents to be shared between both animals.

Parabiosis was used to investigate obesity and diabetes in mice. Two mice lines Db (diabetic) and Ob (obese) have the same overeating phenotype. This is caused by a recessive mutation db^- and ob^- respectively. Figure 1 shows the results of parabiosis between wild-type, diabetic, and obese mice.

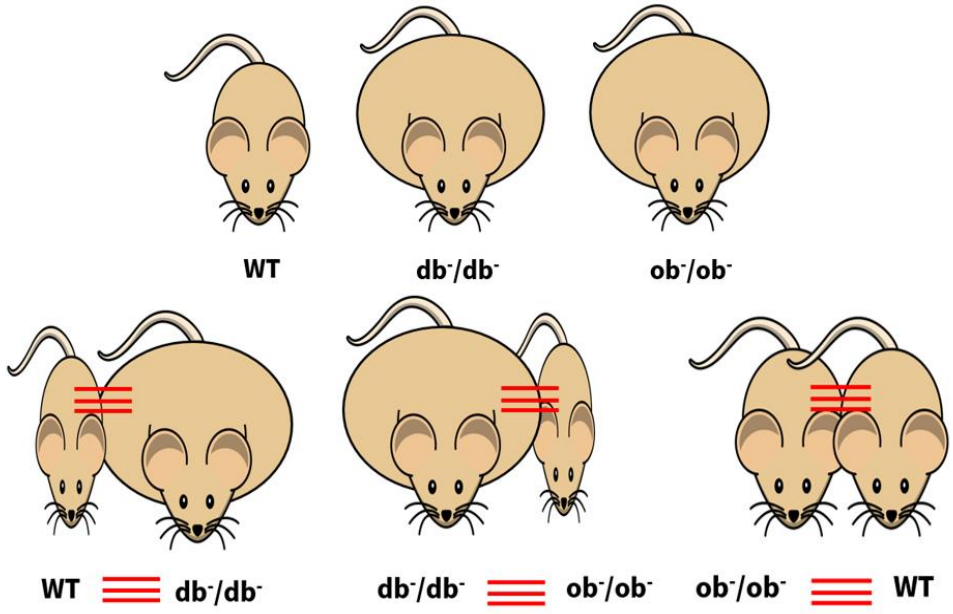


Figure 1: Results of Parabiosis between WT, db^-/db^-, and ob^-/ob^- mice.
Figure is to scale.

Q1. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The *ob* gene codes for a hormone that directly causes weight loss.
B. The *ob* gene causes the production of a satiety factor.
C. Production of satiety factor in WT mice will prevent starvation in ob^-/ob^- mice.
D. ob^-/ob^- mice produce excessive amounts of a substance that reduces appetite.

Q2. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The *db* gene codes for a hormone receptor that directly causes weight loss.
B. We can deduce that the *db* gene definitely codes for leptin.
C. If db^-/db^- mice were to be parabiosed with ob^-/ob^- mice and WT mice together (triple parabiosis), db^-/db^- mice will overeat.
D. If db^-/db^- mice were to be parabiosed with ob^-/ob^- mice and WT mice together (triple parabiosis), ob^-/ob^- mice will starve.

Q3. The two genes are not linked. What is the expected F_2 phenotypic ratio if *ob/ob* db^-/db^- mice are crossed with ob^-/ob^- *db/db* mice? Leave the ratio in its simplest form. **(30 points)**
(Enter a whole number in each row.)

Behaviour	**Ratio**
Starving behaviour	
Normal eating behaviour	
Overeating behaviour	

In a different experiment to determine the factors affecting ageing in mice, you performed a parabiosis experiment with two mice surgically joined at the abdomen. You investigated the rates of methylation of the chromatin and telomere lengths in mice in parabiosis. Figures 2 and 3 show the results.

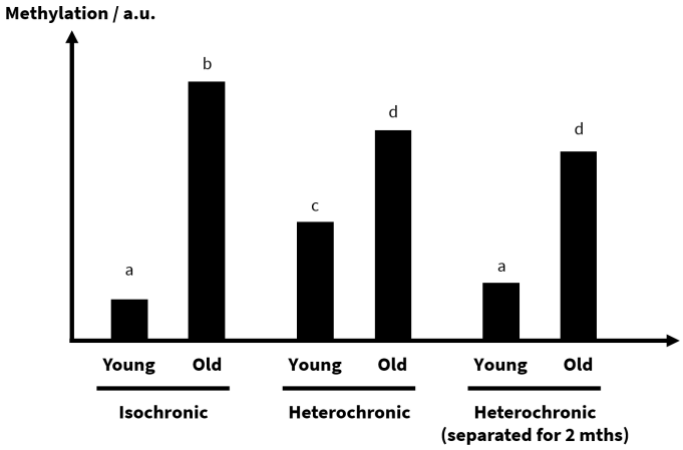


Figure 2: Degree of methylation in each mice. “Isochronic” and “Heterochronic” mice had their data taken while they were still in parabiosis for 1 week. “Heterochronic (separated for 2 months) had their data taken after 2 months of surgical separation from parabiosis.

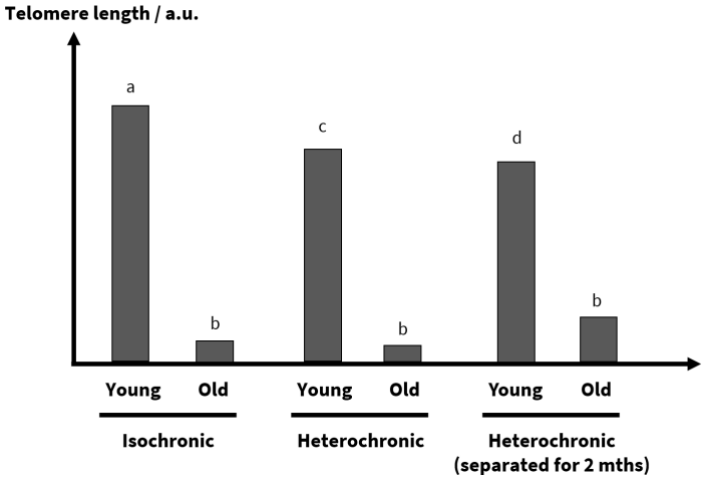


Figure 3: Telomere Length in each mice. “Isochronic” and “Heterochronic” mice had their data taken while they still in parabiosis for 1 week. “Heterochronic (separated for 2 mths) had their data taken after 2 months of surgical separation from parabiosis.

Q4. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. An “old-age” factor that upregulates DNA methyltransferase is produced by the old and circulates in the bloodstream.
B. A “youthful factor” that upregulates DNA methyltransferase is quickly degraded in old mice.
C. Telomerase is secreted in bloodstream of young mice.
D. There are relatively higher rates of mitosis in young mice than old mice.

Answers and Explanations

Analysing the experiment

Parabiosis links the circulatory system of two mice together. Hence any hormones produced by one mouse will circulate in the circulatory system to the other mouse too.

We observe three different phenotypes: Normal, thin, and large. In the first parabiosis experiment, the WT mouse became thin, implying that the db^-/db^- mouse produced some satiety factor that signalled to the wild-type mice to stop eating. Thus the WT mouse believed that it was full and stopped eating, hence becoming thin (starving). Meanwhile, the db^-/db^- mouse remained large, implying that it did not stop eating. Hence, this suggests that the ***db*$^-$*/db*$^-$ mouse is insensitive to this satiety factor** and hence continued to overeat. **Therefore, the *db* gene codes for the receptor that binds to the satiety factor.**

In the second experiment, we see a similar result, with the ob^-/ob^- mouse turning thin. This also suggests that the reason the ob^-/ob^- mouse turns large is not due to insensitivity to this satiety factor.

In the third experiment, the ob^-/ob^- mouse became normal sized as opposed to large, suggesting that WT mice produced some satiety factor that signalled to the ob^-/ob^- mouse that it has eaten sufficient and to stop overeating. Hence, we can deduce that ***ob*$^-$*/ob*$^-$ mouse likely is sensitive to this satiety factor** as it stops eating in both experiments 2 and 3, but it is **unable to produce this satiety factor**, causing it to be unable to recognise when it has eaten sufficient and thus overeat. **Therefore, the *ob* gene codes for the satiety factor, which is now known as leptin.**

Q1.

Answer: **FTFF**

Explanation:

A. The satiety factor does not directly cause weight loss. It simply signals to the brain that there is sufficient food consumed. It is the overproduction of the satiety factor that will signal to the brain to stop eating even when insufficient food is consumed, causing the organism to lose weight.
B. Explained above.
C. ob^-/ob^- mice do not starve.
D. ob^-/ob^- mice underproduce the satiety factor that reduces appetite.

Q2.

Answer: **FFTT**

Explanation:

A. The receptor does not directly cause weight loss. It simply allows signal transduction by binding to the satiety factor to signal to the brain to stop eating. It is the overproduction of the satiety factor that will signal to the brain to stop eating even when insufficient food is consumed, causing the organism to lose weight.
B. While we now know that the satiety factor is leptin, we cannot directly deduce it.
C. With all three mice parabiosed, an excess of the satiety factor will be produced. However, db^-/db^- mice are insensitive to the satiety factor and will thus continue to overeat.
D. With all three mice parabiosed, an excess of the satiety factor will be produced. Since ob^-/ob^- mice are sensitive to the satiety factor and there is an excess of it, the ob^-/ob^- mice will stop eating before it has consumed sufficient food and will thus starve.

Q3.

Answer: **0, 9, 7**

Explanation: The F1 generation of mice will have genotype $ob^-/ob\ db/db^-$. We can thus construct a dihybrid Punnett square as seen below:

	ob db	*ob* db^-	ob^- *db*	ob^- db^-
ob db	*ob ob db db*	*ob ob db* db^-	*ob* ob^- *db db*	*ob* ob^- *db* db^-
ob db^-	*ob ob db* db^-	*ob ob* db^- db^-	*ob* ob^- *db* db^-	*ob* ob^- db^- db^-
ob^- *db*	*ob* ob^- *db db*	*ob* ob^- *db* db^-	ob^- ob^- *db db*	ob^- ob^- *db db*
ob^- db^-	*ob* ob^- *db* db^-	*ob* ob^- db^- db^-	ob^- ob^- *db* db^-	ob^- ob^- db^- db^-

Orange: Normal phenotype | Blue: Overeating phenotype

- *ob ob db db* mice have normal phenotype.
- ob^- ob^- *db db* mice do not produce the satiety factor, thus they will overeat.
- *ob ob* db^- db^- mice have insensitive satiety factor receptors, thus they will overeat.
- ob^- ob^- db^- db^- mice do not produce the satiety factor and have insensitive satiety factor receptors, thus they will overeat.

Hence the ratio is 9 Normal : 7 Overeating.

Another way to approach this question is to recognise that as long as the mouse is homozygous for either gene, it will overeat. Hence this is a case of duplicate recessive epistasis with a known ratio of 9:7.

Q4.

Answer: **TFFT**

Explanation:

A. This factor is produced by the old mice so during parabiosis of young and old, the young mice had an increase in methylation. The increase in methylation likely comes about due to upregulation of DNA methyltransferase.
B. It does not make sense for a “youthful factor” to upregulate DNA methyltransferase and be produced in the young mice with low methylation levels, and yet the old mice degrades it.
C. Old mice had no change in the telomere length even during parabiosis implying that telomerase is not secreted by young mice and transported in the bloodstream.
D. After 2 months of separation, the telomere length fell for the young mice but did not change for the old mice implying that more cell division occurred in the young mice causing the telomere to be shortened.

P12: How should I choose my flowers?

(130 points)

Jen is trying to buy some flowers for her friend's dance concert. Her friend loves flowers and gives her a specific set of instructions on what flower to get.

Her friend, Lily, gave the following set of instructions:

1. The flower needs to be zygomorphic
2. The flower needs to have an inferior ovary
3. The flower belongs to a monocotyledonous plant
4. The flower is unisexual

Q1. Which of the following plants have flowers corresponding to the above set of instructions? **(20 points)**
(Select the correct option.)

A. Valerian herb
B. Sweet pea
C. Antirrhinum
D. Chayote
E. Coconut
F. Sack-shaped catasetum

Jen also noticed that some of the flowers she saw at the florist bloomed in clusters. After some googling, she discovers that such a blooming pattern is known as inflorescence. There is determinate and indeterminate inflorescence. For determinate inflorescence, the oldest flower is located at the tip and younger flowers bud moving down the axis towards the base. For indeterminate inflorescence, the oldest flower is located at the base and younger flowers bud moving up the axis towards the tip.

Figure 1 shows several flowers with different types of inflorescences.

Figure 1: Flowers with different inflorescences.
(Left): Flower 1. (Middle): Flower 2. (Right): Flower 3.

Q2. Based on Figure 1, indicate whether each type of inflorescence is determinate or indeterminate by entering *D* if the flower has determinate inflorescence and *N* if the flower has indeterminate florescence. **(30 points)**
(Enter "D" or "N" to each row.)

Flower	**Type of inflorescence (D or N)**
1	
2	
3	

There are several types of indeterminate inflorescence as follows:

- **Spike**: The flowers are attached directly to the axis without being attached to a pedicel
- **Capitulum**: The flowers are attached directly on a broad, flat peduncle, making the inflorescence seem like a single flower
- **Raceme**: The flowers are each attached to a pedicel, which is in turn attached to the axis.
- **Corymb**: The pedicels of the lower flowers are longer the pedicels of the upper ones, making the overall appearance of the inflorescence to be flat
- **Umbel**: The flowers are attached to pedicels, with each pedicel growing from about the same point at the tip of the peduncle, giving an umbrella-like shape for the inflorescence.

Figure 2 shows several flowers with different types of indeterminate inflorescences.

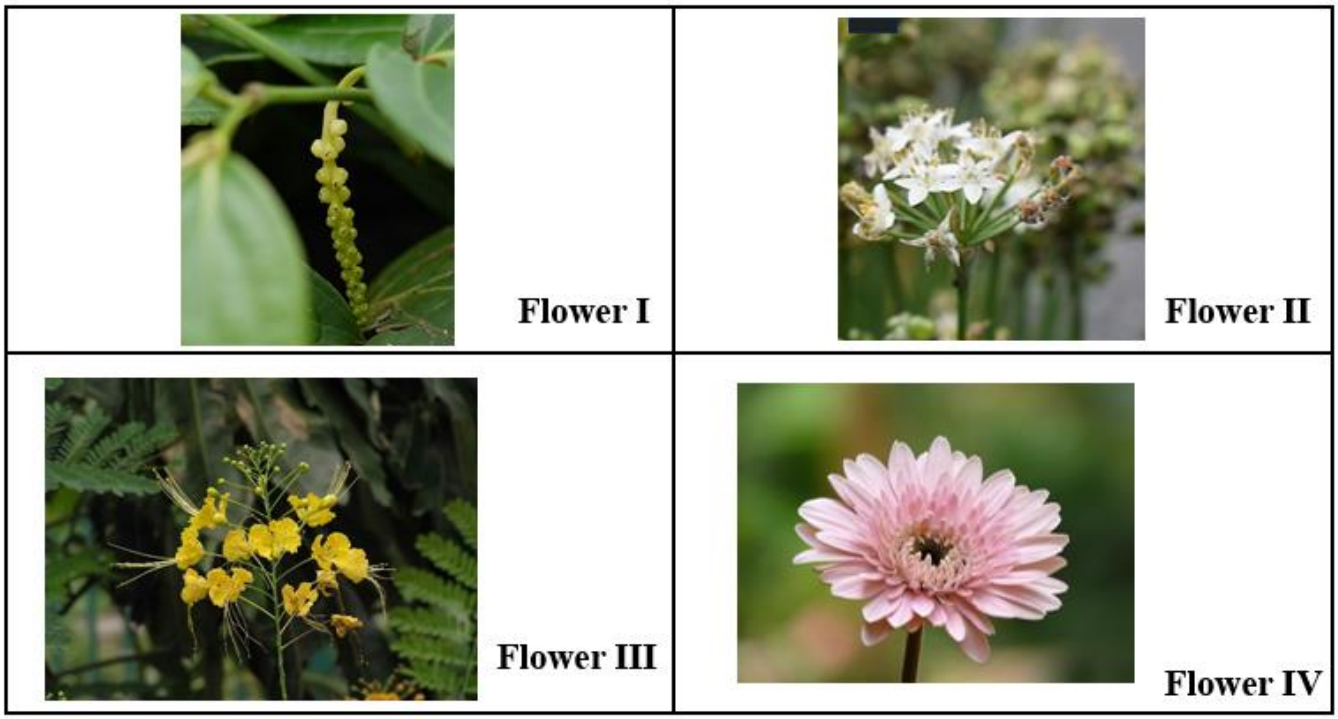


Figure 3: Flowers with different indeterminate inflorescences

Q3. Match the type of indeterminate inflorescence (1-5) to the flowers in Figure 2. **(40 points)**
(Enter a number to each row.)

1. Spike
2. Capitulum
3. Raceme
4. Corymb
5. Umbel

Flower	**Type of indeterminate inflorescence (1-5)**
I	
II	
III	
IV	

Jen also happened to be buying flowers for her friend Jade, who was an eccentric biologist. Jade asked for a bouquet of flowers comprising 1 stalk of Flower 1, 2 stalks of Flower 2, 3 stalks of Flower 3 and 4 stalks of Flower 4 and represented each flower using cryptic floral formulae as seen in the table below.

Flower 1	**Flower 2**
$\% \, ♂♀ \, K_5 \, C_5 \, A_{5-\infty} \, \underline{G}_1$	$\oplus \, ♂♀ \, K_4 \, C_4 \, A_{2+4} \, \underline{G}_{(2)}$
Flower 3	**Flower 4**
$\oplus \, ♂♀ \, P_{3+3} \, A_{3+3} \, \underline{G}_{(3)}$	$\oplus \, ♂♀ \, K_{(5)} C_{(5)} A_5 \, \underline{G}_{(2)}$

Jen entered a floral shop and was presented with a plethora of options. Jen was confused about which flowers she had to buy, could you help her?

Q4. Match Flowers 1 to 4 to the flower that they represent (A-H) in Figure 3. **(40 points)**
(Match the correct letter to the correct row.)

Flower	**Flower as represented in Figure 4 (A-H)**
1	
2	
3	
4	

A	B
	
C	**D**
	

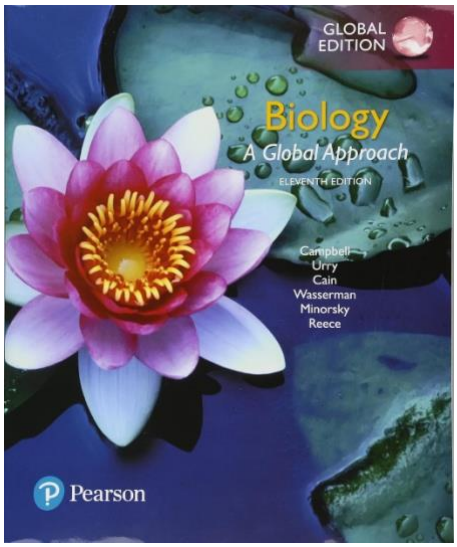
E	F
	
G	**H**
	

Figure 3: Different flowers

Answers and Explanations

Q1.

Answer: **F**

Explanation:

A. Valerian herb is a dicotyledon.
B. Papaya flowers have radial symmetry.
C. Antirrhinum is a dicotyledon.
D. Chayote is dicotyledonous.
E. Coconut has radial symmetry and unisexual flowers.
F. Phalaenopsis, which is an orchid, fits all the criteria.

Q2.

Answer: **N, D, D**

Explanation: To deduce if the inflorescence is determinate or indeterminate, one can look at the flower at the tip of the inflorescence. For indeterminate inflorescence, the flower at the tip is the newest and is hence shown as a bud/small flower in images (i.e. in flower 1). For determinate inflorescence, the flower at the tip is the oldest and hence is fully bloomed/largest in size (i.e. flowers 2 and 3).

A. *Arabidopsis thaliana*
B. *Stellaria media*
C. *Ranunculus acris*

Q3.

Answer: **1, 5, 3, 2**

Explanation:

A. The flower has a spike inflorescence since the pedicel is absent and flowers (the small round shaped structures) are directly attached to the axis.
B. The flowers can be seen to form a rough umbrella shape, with each pedicel having roughly the same length (which is how the umbrella shape is formed).
C. The flowers are arranged in a similar shape as 1, however, the inflorescence is raceme instead of spike as pedicels are present.
D. The flower is made up of multiple florets which form what looks like a single flower and is hence a capitulum.

Q4.

Answer: **E, A, F, H**

Explanation:

A. Flower 1 is bilaterally symmetrical which narrows it down to C or E. It also has at least 5 stamens so that leads to E.
B. Flower 2 has 4 petals, so it is A. A total of 6 stamens are also seen.
C. Flower 3 has 2 whorls of 3 petals each, so it is F. A total of 6 stamens are also seen.
D. Flower 4 has 5 fused petals, so it is H.

Credits

Figure 2:

Flower I: Valke, D. (2010, 24 August). *Pokhlem Mirim*. Wikipedia. https://commons.wikimedia.org/wiki/File:Pokhlem_Mirim_(Konkani-_%E0%A4%AA%E0%A5%8B%E0%A4%96%E0%A5%8D%E0%A4%B3%E0%A5%87%E0%A4%82_%E0%A4%AE%E0%A5%80%E0%A4%B0%E0%A5%80%E0%A4%82)_(4956219770).jpg

Flower II: Gr4yleaf. (2017, 30 September). *Allium tuberosum*. Wikipedia. https://commons.wikimedia.org/wiki/File:Allium_tuberosum_one_flowerhead.jpg

Flower III: 阿橋 HQ. (2007, 1 October). *Caesalpinia pulcherrima*. Wikipedia. https://commons.wikimedia.org/wiki/File:%E6%B4%8B%E9%87%91%E9%B3%B3_Caesalpinia_pulcherrima_-%E9%A6%99%E6%B8%AF%E8%BF%AA%E6%AC%A3%E6%B9%96_Inspiration_Lake,_Hong_Kong-_(9216100734).jpg

Flower IV: Jose, J. (2010, 10 January). *Gerbera in Kadavoor*. Wikipedia. https://commons.wikimedia.org/wiki/File:Gerbera_in_Kadavoor.jpg

Figure 3:

Flower A: Darlington, R. (2012, 18 June). *Horse-radish*. Horse-Radish / Horse Radish - Wild Flower Finder. https://wildflowerfinder.org.uk/Flowers/H/HorseRadish/HorseRadish.htm

Flower B: Leidus, I. (2021, 16 May). *Cerastium arvense*. Wikipedia. https://en.wikipedia.org/wiki/Cerastium_arvense

Flower C: Hectonichus. (2013, 4 August). *Salvia transsylvanica*. Wikipedia. https://en.m.wikipedia.org/wiki/File:Lamiaceae_-_Salvia_transsylvanica.jpg

Flower D: Milliken, W., Klitgard, B. and Baracat, A. (2009 onwards). *Neotropikey - Interactive key and information resources for flowering plants of the Neotropics*.

Flower E: Hectonichus. (2008, 7 May). *Spartium junceum*. Wikipedia. https://is.m.wikipedia.org/wiki/Mynd:Fabaceae_-_Spartium_junceum-003.JPG

Flower F: Westmount Florist. (2023, 30 June). *Lily*. https://westmountflorist.com/blogs/flower-dictionary/lily

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P13: Don’t cry over spilt milk, EMMA!

(150 points)

Emma recently learnt about Northern Blot, which is used to detect gene expression by analysis of the amount of mRNA produced by the cell. Her professor has tasked each student to come up with two experiments to show their understanding of Northern Blot.

Emma loves milk a lot and always brings a bottle of milk with her at all times. As she carried her milk bottle (Figure 1) into the lab, she realised that milk contains much lactose, which is a disaccharide found in large quantities in milk, and that she had recently read about the *lac* operon. She could possibly perform a Northern Blot experiment on it!

Figure 1: Emma’s Milk Bottle

The *lac* operon in *E. coli* encodes for β-galactosidase, which breaks down lactose into glucose and galactose. Emma thus created several *E. coli* mutant cells and allowed them to grow in media containing lactose but no glucose for 24 hours. She then performed Northern Blot against β-galactosidase mRNA.

After performing the Northern Blot, she noticed that there are three different intensities of the band produced: None, Low, and High.

Q1. Help Emma match the following strains of *E. coli* with the intensity of the band (1-3) expected after the Northern Blot. **(60 points)**

(Enter the correct number to the correct row.)

1: None

2: Low

3: High

***E. coli* strain**	**Intensity of band (1-3)**
Normal strain	
Strain with nonsense mutation in *lacI*	
Strain with a permanent binding of allolactose to the repressor, and glucose is added to the cell	
Strain with a premature stop codon in *lacZ*	
Strain with inactive permease	
Strain with a mutation in the CAP-binding site such that CAP can no longer bind to it	

As she finished her experiment, she decided to reward herself with a sip of milk. Sipping on her milk bottle (Figure 1), she realised babies drink milk too. Maybe she can perform an experiment on morula cells!

Emma decided to extract cells from the adrenal cortex, adrenal medulla, embryo morula, and a malignant tumour. She performed Northern Blot by isolating the mRNAs produced by each of the cells and then carried out gel electrophoresis separately using radioactive probes specific to five sequences of mRNA (A - E). These mRNA sequences code for calcitonin, epinephrine, histone H1, P53 protein, and telomerase, but not necessarily in that order. Unfortunately, Emma was careless and spilt milk on her paper, covering up to what the mRNA sequences and cells correspond. You told her not to cry as there is no use crying over spilt milk, and instead promised her that you would use her results to determine what the proteins added were.

The results are shown in Figure 2.

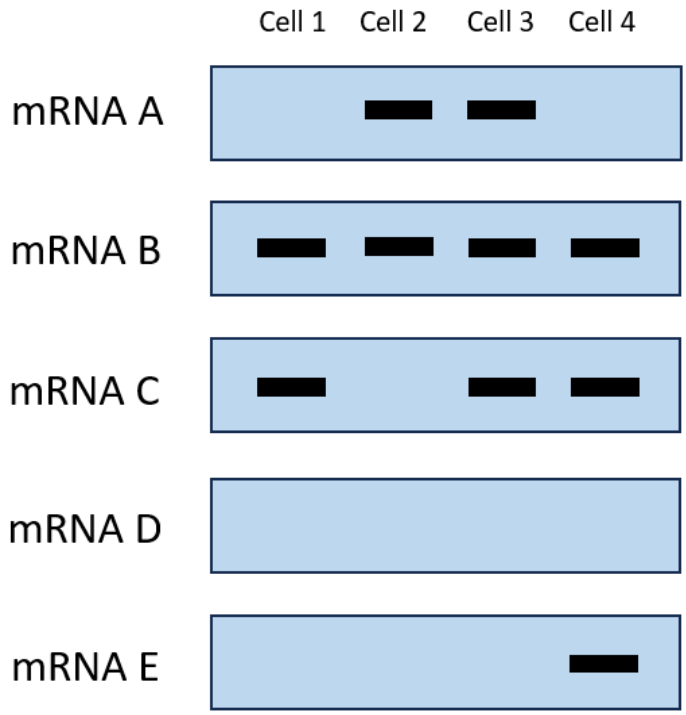


Figure 2: Gel electrophoresis using probes complementary to five different mRNA which code for proteins

Q2. Help Emma match the mRNA letters (A, B, C, D, E) to the protein of which they code for. **(50 points)**

(Match the correct letter to the correct row.)

Identity of Protein	**Letter**
Calcitonin	
Epinephrine	
Histone H1	
P53	
Telomerase	

Q3. Help Emma match the cell numbers (1, 2, 3, 4) to their correct identities. **(40 points)**

(Match the correct number to the correct row.)

Identity	**Number**
Adrenal Cortex Cells	
Adrenal Medulla Cells	
Morula Cells	
Tumour Cells	

Hope that this is a poignant reminder not to bring food and drinks into the lab and that Emma would not do it again.

Answers and Explanations

Q1.

Answer: **3, 3, 2, 3, 1, 2**

Explanation:

In the presence of lactose, allolactose will bind to the repressor, preventing it from binding to the operator. Hence the operon will be **active** and β-galactosidase mRNA will be produced in large amounts.

A. Normal strain will have high levels of mRNA produced.
B. A nonsense mutation in *lacI* will result in a truncation of the repressor polypeptide produced, hence the repressor can no longer bind to the operator. However, the repressor was not bound to the operator in the first place, so the result is the same as that with the normal strain.
C. Allolactose is permanently bound to the repressor, so the operon is left active. However, glucose is added to the cell, so there is less cyclic AMP (cAMP) present to bind to the catabolite activator protein (CAP), so there is less binding of CAP to the CAP-binding site, so the operon is active but is not upregulated, so the amount of mRNA produced will be at an intermediate level.
D. A premature stop codon in *lacZ* will result in the protein produced by *lacZ* to be truncated. However, the entire mRNA will still be produced at high amounts, so the result is still the same.
E. With inactive permease, lactose cannot enter the cell. Thus, the repressor remains bound to the operator, turning the operon inactive and thus mRNA is not produced.
F. CAP can no longer bind to the CAP-binding site so the operon is not upregulated despite being active. Hence, there will be a band of intermediate intensity.

Analysing the EMSA assay results

We need to determine which cells produce which proteins and hence their corresponding mRNAs will be produced in their cells and will appear as a band on the Northern Blot. All four cells contain chromosomes which require histones to allow the chromatin to be wound. Hence, all four cells should produce Histone H1 mRNA. Hence **mRNA B belongs to Histone H1**.

Next, calcitonin opposes the effect of the parathyroid hormone and reduces blood calcium levels. It is produced by the parafollicular cells (C-cells) of the thyroid gland, so none of the four cells should be producing it. Hence, **mRNA D belongs to calcitonin**.

P53 protein should be produced in all cells except cancer cells as *p53* is a tumour suppresor gene which helps regulate the cell cycle. The loss of this protein in cancer cells allows for uncontrolled cell division as the cell cycle is dysregulated. Hence, **mRNA C belongs to P53 protein** and **cell 2 is a cancer cell**.

Next, telomerase is produced in cancer cells hence extending the Hayflick limit as the telomeres are continually elongated, so the cells can undergo uncontrolled mitosis. Telomerase is also produced in morula cells to maintain cellular length and cellular immortality. Hence, **mRNA A belongs to telomerase**. Since cell 2 is a cancer cell, **cell 3 is a morula cell**.

Lastly, epinephrine is produced in adrenal medulla cells and not the adrenal cortex cells. By the process of elimination, **mRNA E belongs to epinephrine**. **Cell 4 is thus an adrenal medulla cell**, and **cell 1 is an adrenal cortex cell**. While it is true that some cancer cells may also overproduce epinephrine if it is part of a tumour called pheochromocytoma, it is extremely rare. In addition, there is no other combination of correct answers. Hence, we can assume that epinephrine is not produced in the cancer cell.

Q2.

Answer: **D, E, B, C, A**

Explanation: See above.

Q3.

Answer: **1, 4, 3, 2**

Explanation: See above.

P14: Hereditary Cancer

(110 points)

Cancer is one of the leading causes of death and disability in the world. In particular, breast cancer is also the most common cancer in women in Singapore. One of the common genes responsible for breast cancer, BRCA, can be inherited.

Below is the family tree of a family with a history of breast cancer. Assume that there are no *de novo* mutations in the BRCA gene during gametogenesis by any individual in the following pedigree.

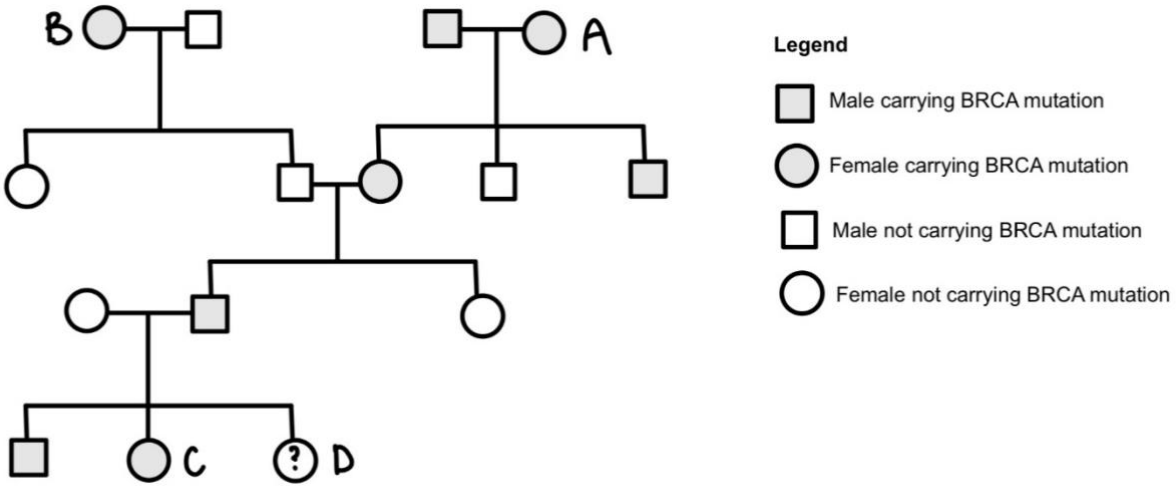


Figure 1: Pedigree

Q1. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. From the pedigree, individual A definitely suffered from breast cancer.
B. Given that individuals A and B had different mutations in the BRCA gene, the mutation in individual C is the same as the mutation in individual B.
C. It is unknown if individual B inherited the BRCA mutation from her parents.
D. The likelihood of individual D having breast cancer is 50%.

Individuals A and B both have breast cancer. Their information is listed in the table below.

Patient A: Stage 3 breast cancer, HER2+, 1081delG BRCA1 mutation, Oestrogen and Progesterone receptor negative **Patient B:** Stage 2 breast cancer, HER2-, 4265delCT BRCA2 mutation, Oestrogen receptor positive

Q2. There are many different founder mutations for the BRCA1/2 genes, including BRCA1 founder mutation (1081delG) from South China and BRCA2 mutation (4265delCT) in the Philippines. Given that BRCA1/2 genes are both tumour suppressor genes, which of the following treatment(s) could be beneficial for **both** patients A and B? **(20 points)**
(Select all correct options.)

A. Mastectomy
B. PARP inhibitor
C. Herceptin (trastuzumab)
D. Hormone therapy

The BRCA1/2 gene codes for proteins involved in a process named "homologous recombination". Homologous recombination is one of the many methods of DNA repair in our cells to ensure genomic stability. Other DNA repair mechanisms include mismatch repair, nucleotide excision repair, non-homologous end joining and base excision repair.

The descriptions to the 5 different methods of repair are given below:

Code	Name of Repair Method	Repair Mechanism
I	Homologous recombination	Process where an undamaged DNA molecule is invaded by a damaged molecule of identical or very similar sequence. Undamaged DNA is then used as a template for the repair of the damaged DNA via complementary base pairing.
II	Mismatch repair	Process responsible for correcting errors made during DNA replication, hence preventing these mutations from becoming permanent in dividing cells.
III	Nucleotide excision repair	Main process responsible for removing bulky DNA lesions.
IV	Non-homologous end joining	A type of DNA repair which mediates the direct religation of the broken DNA molecule does not require a homologous template for repair of the DNA lesion like in homologous recombination.
V	Base excision repair	Process responsible for removing small base lesions that do not significantly distort the DNA helix structure.

Q3. Match the following DNA lesions to the code of the repair method (I-V) that is most likely responsible for repairing the damage. Use each roman numeral only once. **(50 points)**

(Enter a roman numeral to each row.)

Lesion	**Repair Method (I-V)**
Thymine dimer	
Guanine replaced by thymine due to a cytostatic drug	
DNA interstrand crosslink	
DNA polymerase wrongly adding cytosine instead of adenine	
DNA double-stranded breaks at G_1 phase of cell cycle	

Answers and Explanations

Q1.

Answer: **FFTF**

Explanation:

A. Cancer development requires multiple steps and mutations; carrying the BRCA mutation predisposes one to breast cancer but does not guarantee development of breast cancer.
B. Mutation in C came from A. If the son of B did not carry the BRCA mutation, the mutation from B would not have been able to be passed on to C, who is the great granddaughter of B. Instead, the mutation from A was passed down to A's daughter, followed by A's grandson and eventually C.
C. B might have the BRCA mutation due to spontaneous mutations during processes such as gamete development, since we do not have information about B's parents, we cannot be sure if the BRCA mutation in B came from them.
D. The likelihood of D carrying BRCA mutation is 50%, but that is not equivalent to the likelihood of D having breast cancer.

Q2.

Answer: **A, B**

Explanation:

A. Stage 2 cancer is still localised while stage 3 cancer is larger with possibly some local invasion and to surrounding lymph nodes. Both can be treated with mastectomy as the cancer is still generally located within the breast region.
B. Both individuals have BRCA mutations, and can hence be treated using the PARP inhibitor which is used to treat BRCA mutation breast cancers. Both the BRCA genes and PARP proteins are involved in DNA repair. When cancer cells have loss of function mutation for BRCA gene and also have its PARP proteins inhibited, it is more likely to die as DNA damage cannot be repaired.
C. Herceptin is only used to treat HER2+ cancers and thus will not work on individual B who is HER2-.
D. Hormone therapy is used for hormone receptor positive cancers and thus will only be effective for individual B but not A.

Q3.

Answer: **III, V, I, II, IV**

Explanation:

A. Thymine dimer is bulky, hence has to be removed via nucleotide excision repair.
B. Mutation (not during DNA replication) and is small as only a single nucleotide is replaced.
C. Interstrand crosslinks are complex DNA damages which are fixed by homologous recombination.
D. Error occurred during replication and hence is fixed by mismatch repair.
E. NHEJ is the primary DNA double strand break repair mechanism during the G1 phase of the cycle and not homologous recombination.

P15: Golden Apple Archipelago

(210 points)

The Golden Apple Archipelago is a tropical region near the coast of *Biotropica* with many rainforests. The archipelago is named after the **golden apple tree**, which is the most common native tree in the region, dominating most of the rainforests. The golden apple tree is part of the Myrtaceae family, growing to variable heights (from shrubs to tall trees) while being able to grow at variable elevations, temperatures and rainfall intensity.

The **sunsettia** is a small, shade-tolerant tree that was introduced to the Golden Apple Archipelago more than 200 years ago. While also from the Myrtaceae family, the sunsettia tree is shorter at its maximum height compared to the golden apple tree.

The sunsettia is considered an invasive species and has spread throughout some of the rainforests in the Golden Apple Archipelago. Figure 1 shows a diagrammatic map of the region and the relative densities of the sunsettia, dated 2025.

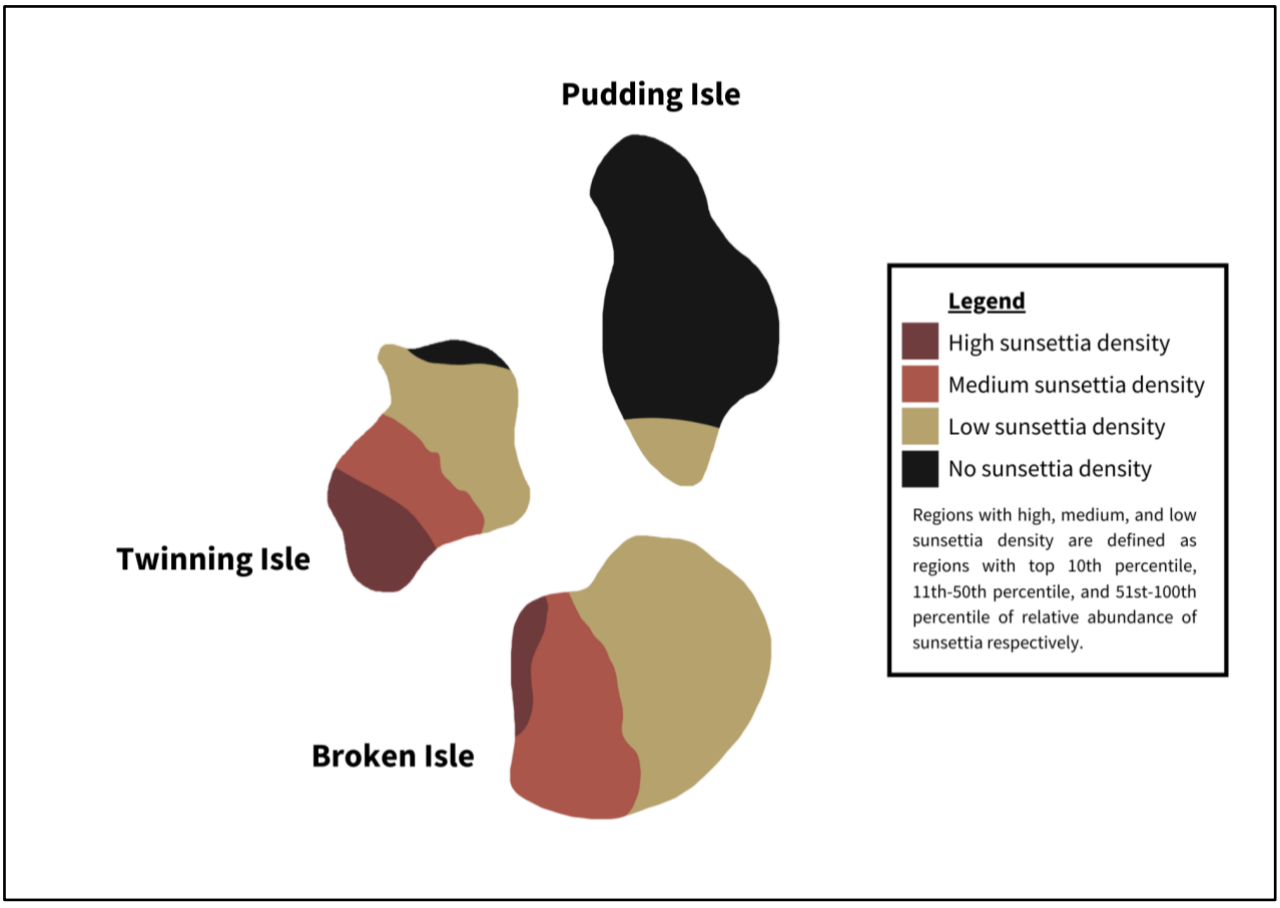


Figure 1: Map of the Golden Apple Archipelago, dated 2025.

In 2025, a team of ecologists was commissioned to study the population dynamics of the sunsettia in the Golden Apple Archipelago for **16 years (2025 to 2040 inclusive)**. The team marked out five randomly selected replicate study plots of 0.25 ha on each of the three islands, selecting intact golden apple forest regions with **low sunsettia density**. All study plots have similar initial densities of the sunsettia with a range of stem diameters represented.

Analysis I: Population counts

At the start of 2025, in each of the study plots, all sunsettia stems with a diameter at breast height (DBH) of at least 2 cm were tagged. At the start of every year, new eligible sunsettia recruits were tagged, while dying sunsettia stems were noted down and no longer included in the study population from that year onward.

A portion of the collected data is shown in the table below.

	2025	**2026**	**2027**	**2028**
Population count on Twinning Isle (stems/ha)	13,503	13,763	14,040	15,558

Ecologists use annual population counts to study the annual population growth of a population, denoted as **lambda**. The lambda of a year is the ratio of population sizes between the following year and the current year.

$$\lambda = \frac{N_{t+1}}{N_t}$$

Q1. Calculate the average lambda of the sunsettia population at the study site on Twinning Isle from 2025 to the start of 2028. **(20 points)**
(Enter your answer correct to 3 s.f.)

Q2. Use your calculated average lambda to estimate the sunsettia count in 2029, in stems/ha. **(10 points)**
(Enter your answer correct to the nearest whole number.)

Q3. In a 0.1 ha subset of a Twinning Isle study plot, 34 sunsettia plants were newly tagged in 2026. Estimate the *per capita* death rate of the sunsettia in 2025, in deaths/stem. **(20 points)**
(Enter your answer correct to 3 s.f.)

Figure 2 shows the density of sunsettia stems between 2025 and 2040 on each isle based on the information gathered from the study plots.

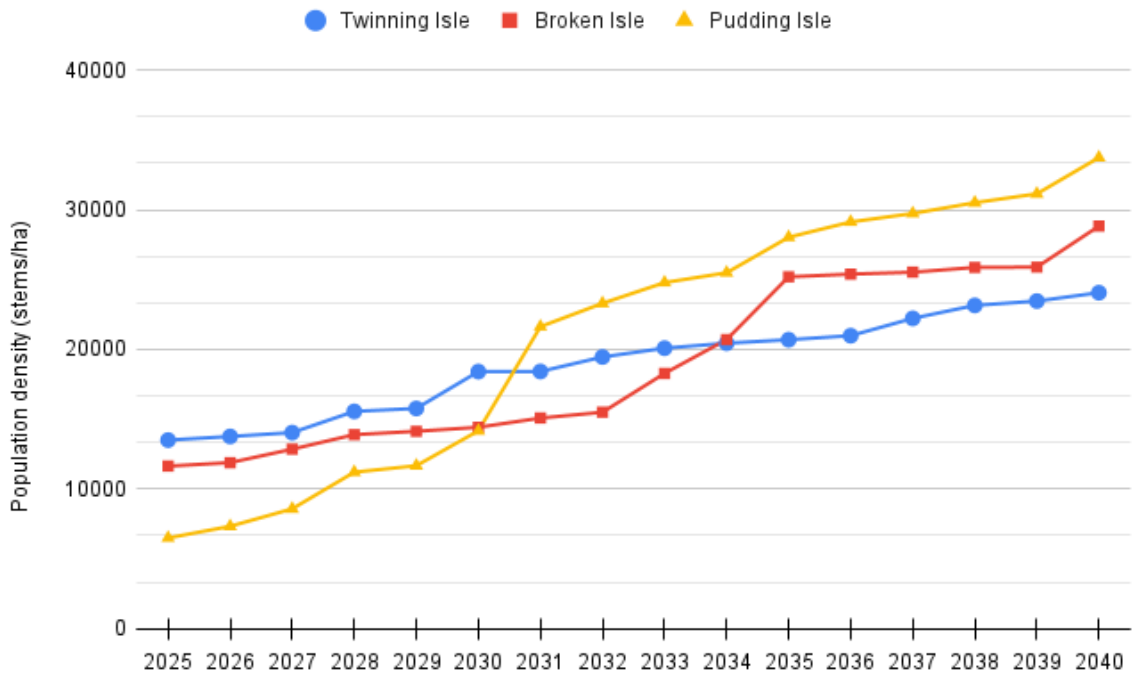


Figure 2: Density of sunsettia stems per year.

Q4. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The calculated lambda (λ) will increase if the population size data was given in stems/km^2.
B. The data represented in Figure 2 indicates an overall increase in relative abundance of the sunsettia in all three study sites from 2025 to 2040.
C. Figure 2 suggests that Twinning Isle has the greatest environmental influences that hinder sunsettia growth out of the three isles.
D. A similar gradient in the graph of population density per year, such as that of the Pudding Isle sites from 2036 to 2039 in Figure 2, indicates an approximately constant lambda.

Analysis II: Types of recruitment

Previous studies suggested two classifications of sunsettia recruitment that increases the sunsettia population in the Golden Apple Archipelago.

Table 2: The two types of sunsettia recruitment

Shoot recruitment	Rooted shoots that originate from seed dispersal.
Sprout recruitment	Above-ground sprouts that arise from established stems.

During each data collection period, when tagging each new eligible sunsettia recruit, the ecologists noted down the corresponding method of recruitment. Figure 3 shows the mean density of sunsettia stems across all study sites differentiated by the method of recruitment.

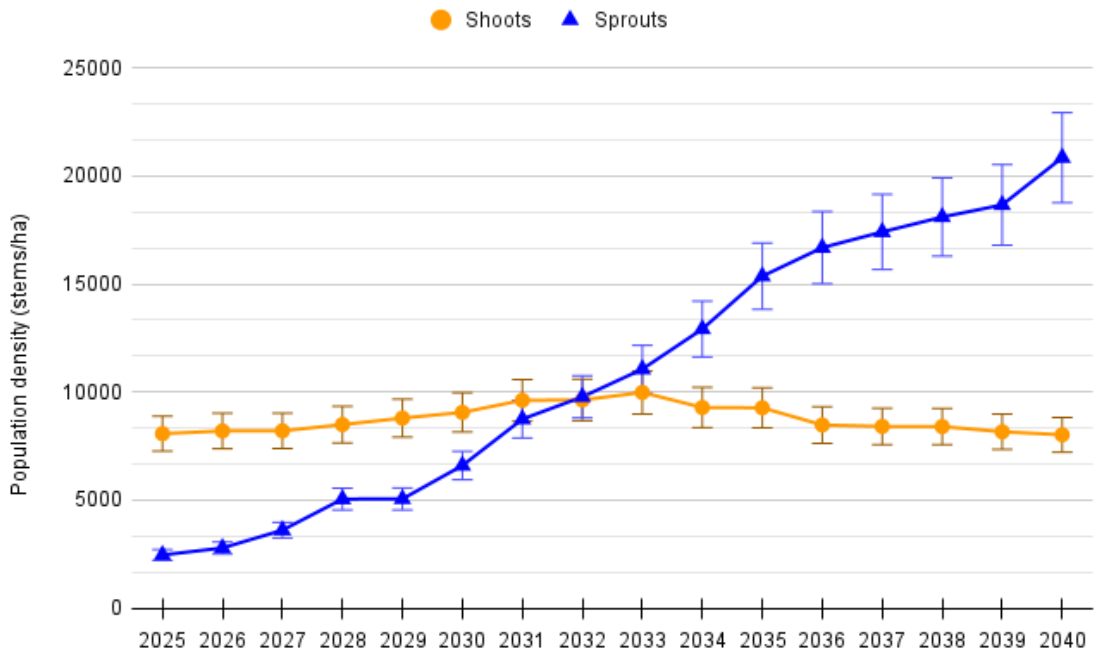


Figure 3: Mean density of shoots and sprouts across all study sites.

Q5. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. In a golden apple forest region on Pudding Isle that reported the first signs of sunsettia invasion in 2040, the sunsettia stems are likely to be shoots rather than sprouts.
B. The average lambda for sunsettia shoots is approximately 0.
C. If there is evidence that sunsettia sprouts have lower flowering rates than sunsettia shoots, then the ratio of sunsettia sporophytes to gametophytes increases across the study sites from 2025 to 2040.
D. The rate of photosynthesis of a randomly selected sprout is greater than the rate of photosynthesis of a randomly selected and equally sized and aged shoot.

Analysis III: Properties of the sunsettia

The sunsettia is a small, shade-tolerant tree that was introduced to the Golden Apple Archipelago more than 200 years ago. Even though its fruits are fleshy and edible, it quickly rots after ripening, subjecting the islands to several pest infestations throughout a sunsettia-dominated forest.

The sunsettia tree is apomictic and polyploid. It utilises two main methods of recruitment: seed dispersal and adventitious shoot development. Sunsettia seeds are mainly dispersed by wild pigs that feed on the sunsettia fruit.

Table 3: Types of spatial distribution.

Interacting with golden apple trees:	**Random**	**Aggregated**	**Uniform**
Independent	*A*	*B*	*C*
Attracted	*D*	*E*	*F*
Repulsed	*G*	*H*	*I*

Q6. Indicate the most likely spatial distribution of sunsettia stems at the study sites in 2025 and 2040 by entering a letter from Table 3 that represents the respective spatial distributions to each row. If you think that none of the distributions in Table 3 represent the most likely distribution, enter *J*. **(20 points)**
(Enter the correct letter to each row.)

Year	**Distribution (A-J)**
2025	
2040	

Q7. The polyploidy of the sunsettia is an evolutionary trait that was traced back to 500 years ago. Currently, throughout the world, diploid sunsettias have been outcompeted by polyploid sunsettias. Which of the following statements are possible reasons for this phenomenon? **(40 points)**

(Select all correct options.)

A. Polyploid sunsettias have greater genetic diversity than diploid sunsettias.
B. Polyploid sunsettias have a more efficient regulation of gene expression compared to diploid sunsettias.
C. Polyploid sunsettias have a higher rate of beneficial mutations than diploid sunsettias.
D. Polyploid sunsettias exhibit more extensive epigenetic modifications for more enhanced phenotypic plasticity than diploid sunsettias.
E. Polyploid sunsettias contain more genes with functions that make polyploid sunsettias fitter than diploid sunsettias.
F. Polyploid sunsettias carry fewer deleterious alleles, which makes them fitter than diploid sunsettias.
G. Polyploid sunsettias have more advanced mechanisms for repairing DNA damage compared to diploid sunsettias.
H. Polyploid sunsettias have a more accurate process of chromosome segregation than diploid sunsettias.
I. Polyploid sunsettias benefit more from heterozygote advantages than diploid sunsettias.

Figure 4 shows several diagrams of reproductive structures in plants.

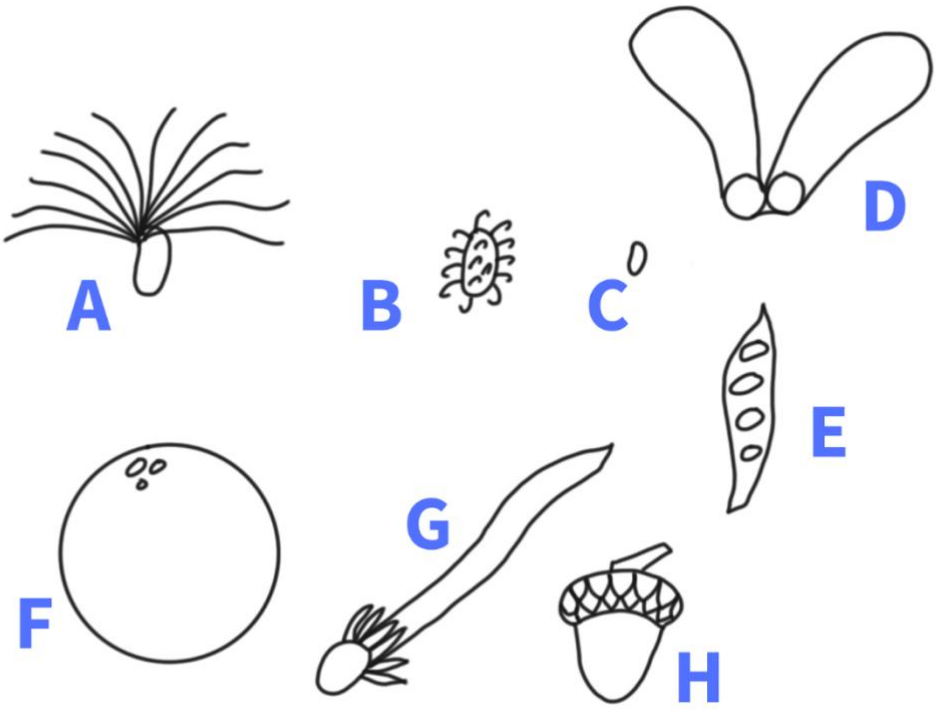


Figure 4: Diagrams of different possible sunsettia reproductive structures.

Q8. Which two reproductive structures most likely belong to the sunsettia plant? **(20 points)**
(Select all correct options.)

A. A
B. B
C. C
D. D
E. E
F. F
G. G
H. H

Answers and Explanations

Q1:

Answer: **1.05**

Explanation: Lambda represents the annual population growth, meaning the average lambda is calculated by summing the individual lambdas of each year (i.e. 2025-2026, 2026-2027, 2027-2028) and dividing the total number of years. Average lambda should not be calculated using only the starting and ending data points (i.e. 2025 and 2028 only).

$$\lambda_{2025} = \frac{13763}{13503} = 1.01925498037$$

$$\lambda_{2026} = \frac{14040}{13763} = 1.02012642592$$

$$\lambda_{2027} = \frac{15558}{14040} = 1.1081196581$$

$$\lambda_{average} = \frac{1.01925498037 + 1.02012642592 + 1.1081196581}{3} = 1.04916702147$$

Hence, the average lambda is **1.05** (3 s.f.).

Q2.

Answer: **16323**

Explanation: We can use our value above to extrapolate the given data and estimate the sunsettia count in 2029.

$$N_{2029} = N_{2028} \times \lambda_{average} = 15558 \times 1.04916702147 = 16323$$

We should not be using the 3 s.f. value of lambda as answered in **Q1** as it is less precise. Moreover, the question stem only asked us to use the calculated lambda value and not specifically the 3 s.f. answer provided.

Q3.

Answer: **0.00592**

Explanation: We can estimate the number of newly tagged sunsettia plants per hectare.

$$N_{birth/ha} = 34 \times 10 = 340$$

Difference in population size $= N_{birth} - N_{death} = N_{2026} - N_{2025}$

$$N_{death/ha} = 340 - (13{,}763 - 13{,}503) = 80$$

$$d = \frac{N_{death/ha}}{N_{2025/ha}} = \frac{80}{13{,}503} = 0.005924609346$$

Hence, the *per capita* death rate of the sunsettia in 2025 is **0.00592** (3 s.f.).

Q4.

Answer: **FFTF**

Explanation:

A. Lambda, representing annual population growth, is a dimensionless ratio between population sizes. As long as the units used are consistent, lambda will not change.
B. While the data shows an overall increase in *absolute* abundance of the sunsettia in all three study sites from 2025 to 2040, it does not contain any data about the abundances of other species which is needed to determine the *relative* abundance of the sunsettia.
C. Twinning Isle shows the greatest density of sunsettia in 2025, but the lowest density in 2040. This suggests greater environmental influences on the Twinning Isle that hinders sunsettia growth, decreasing its capability to recruit new sunsettia plants.
D. Lambda is a ratio between population sizes and is essentially growth *per capita*, meaning the absolute increase in sunsettia plants — represented by the gradient — is not a proxy for lambda, the annual population growth.

Q5.

Answer: **TFTF**

Explanation:

A. The data indicates that for a forest region which has been newly invaded by the sunsettia, there are more sunsettia shoots than sprouts.
B. The average lambda for sunsettia shoots is close to 1, indicating minimal changes in shoot density. A lambda of 0 will indicate local extinction of the species in the study area.
C. The sunsettia, being an angiosperm, houses its gametophytes in the ovule of the flower. If there is evidence that sprouts flower less than shoots, since the data indicates an increase in relative abundance of sprouts compared to shoots, the ratio of sunsettia sporophytes to gametophytes does increase from 2025 to 2040.
D. The rate of photosynthesis of a randomly selected sprout is most likely lower than the rate of photosynthesis of a randomly selected and equally sized and aged shoot. Since sprouts originate from preexisting stems of sunsettia plants, its leaves are more likely to be shielded from sunlight compared to shoots. Seeds are randomly dispersed by animals, and seeds dispersed in regions with little sunlight cover are more likely to grow to maturity, hence a randomly selected shoot is more likely to have high sunlight exposure.

Q6.

Answer: **G, H**

Explanation: Sunsettia shoots (originating from seeds) are randomly dispersed by animals, while sunsettia sprouts are aggregated around pre-existing stems. In 2025, there are more shoots than sprouts, hence the spatial distribution is most likely random. In 2040, there are more sprouts than shoots, hence the spatial distribution is most likely aggregated. Sunsettia plants that are furthest away from then-dominating golden apple trees suffer the least from competition for limited resources and are therefore fitter — indicating a repulsed distribution of sunsettia plants for both 2025 and 2040.

Q7.

Answer: **A, C, I**

Explanation:

A. Polyploid sunsettias have greater genetic diversity than diploid sunsettias because they possess multiple sets of chromosomes that allows for a wider range of genetic variations and combinations. This means polyploid sunsettias adapt to different environmental conditions better than diploid sunsettias.
B. There is no evidence to show that polyploid plants have a more efficient regulation of gene expression than diploid plants.
C. Polyploid sunsettias have a higher rate of beneficial mutations than diploid sunsettias as they possess multiple sets of chromosomes, which increases the overall rate of mutation (rate meaning *per unit time*; this is different from mutation rate where rate means *per nucleotide*).
D. There is no evidence to show that polyploid sunsettias exhibit more extensive epigenetic modifications than diploid sunsettias.
E. Polyploid sunsettias contain more *alleles* than diploid counterparts, but the number of genes remain the same.
F. Polyploid sunsettias may carry *more* deleterious alleles than diploid sunsettias (which can carry at most one for a deadly recessive allele) for sunsettia plants that are still fit for reproduction.
G. There is no evidence to show that polyploid sunsettias have more advanced mechanisms for repairing DNA damage compared to diploid sunsettias.
H. There is no evidence to show that polyploid sunsettias have a more accurate process of chromosome segregation than diploid sunsettias.
I. Polyploid sunsettias have multiple sets of chromosomes, allowing for more allelic variation at each gene locus. This increased genetic variation enhances the likelihood of having beneficial combinations of alleles, which can improve overall fitness through mechanisms such as overdominance, where the heterozygote genotype has a higher fitness than either homozygote genotype.

Q8.

Answer: **B, C**

Explanation: Sunsettias are dispersed by wild pigs. We would expect structures that facilitate animal dispersal, such as B with hook-like structures allowing the seed to hook onto animal fur, or C with its small size to be swallowed by the pig before being excreted. Both structures A and D facilitate wind dispersal. Structures F and G facilitate water-borne dispersal. Structure H is an acorn, which is dispersed by animals that cache acorns (such as squirrels). Pigs do not cache food for the future as they are opportunistic feeders.

Credits

Content reference: Denslow, J. S., Johnson, M. T., Chaney, N. L., Farrer, E. C., Horvitz, C. C., Nussbaum, E. R., & Uowolo, A. L. (2024). Strawberry Guava invasion of a Hawaiian rainforest: Changing Population Patterns. *Biotropica*, *56*(4). https://doi.org/10.1111/btp.13324

P16: Malicious Malicia's Malaria Magnum Opus I

(150 points)

Malaria is caused by a protozoan parasite belonging to the *Plasmodium* genus. Malaria is caused by a protozoan parasite belonging to the *Plasmodium* genus. The genus *Plasmodium* belongs to the phylum Apicomplexa. Nearly all apicomplexans possess the apicoplast, a special type of organelle. Figure 1 shows a *P. falciparum* parasite infecting a red blood cell.

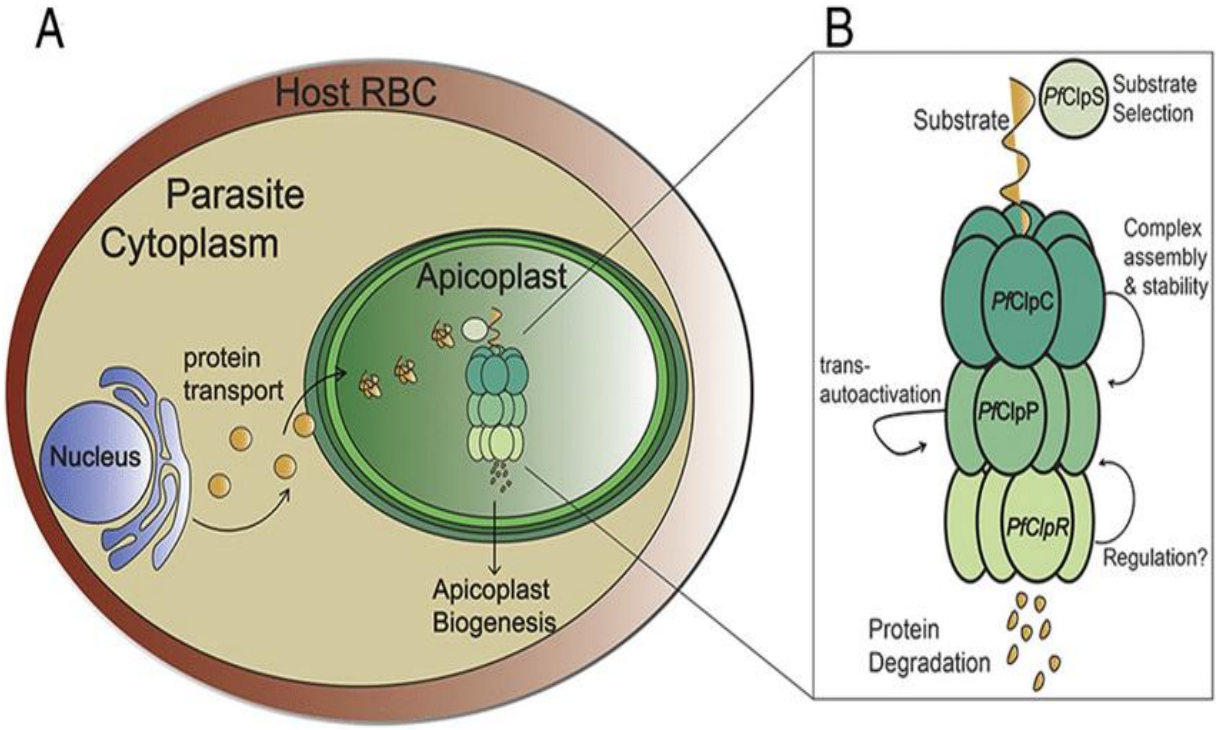


Figure 1: Diagrammatic representation of the *P. falciparum* parasite.

To qualify and stage, flow cytometry is a technique used. The sample containing infected red blood cells is treated and injected into a flow cytometer instrument, where cells pass through a column single file in ideal situations. The column is equipped with a laser which strikes each cell, and the scatter can be analysed to provide information about the physical and chemical characteristics of the cell.

A basic flow cytometry setup is depicted in Figure 2.

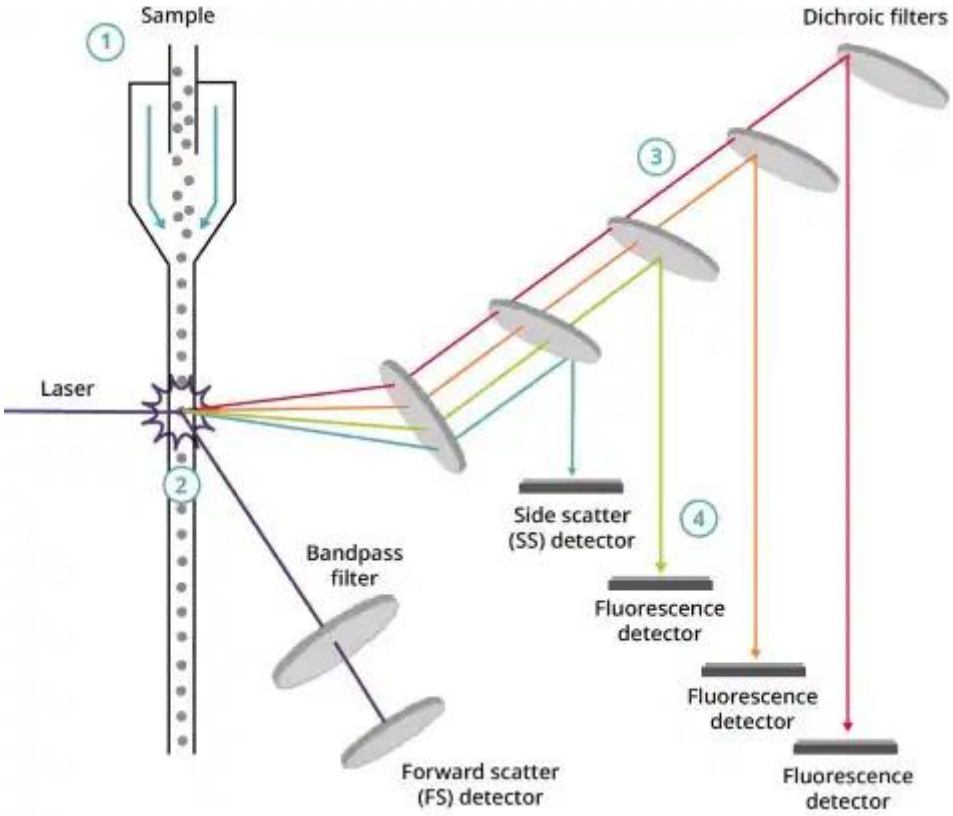


Figure 2: Basic flow cytometry setup

Forward scatter (FSC) and side scatter (SSC) are two parameters measured in flow cytometry. Forward scatter indicates the relative size of the cell, while side scatter indicates the complexity or granularity of the cell.

You are provided with fresh whole blood from a patient infected with *Plasmodium falciparum*. The cell populations present are **infected red blood cells (iRBC)**, **uninfected red blood cells (uRBC)**, **uninfected reticulocytes (uRTIC)** and **white blood cells (WBC)**. Debris is also present. Your task is to calculate the parasitaemia of this patient.

$$Parasitaemia = (\frac{iRBC}{total\ mature\ RBC}) \times 100\%$$

To zone in on your cell population(s) of interest, a process called gating is carried out, where cell populations **not** of interest are identified and excluded in a step-by-step manner — think of it as shortlisting.

The first step is usually to exclude cell debris. After excluding cell debris, the remaining cell populations are plotted with Hoechst vs CD45-APC as the two axes. Hoechst is a stain that the sample is treated with before it undergoes flow cytometry, and a positive Hoechst result indicates the presence of DNA while a negative Hoechst result indicates the absence of DNA. CD45 is an antigen that is found on the surface of nucleated hematopoietic cells.

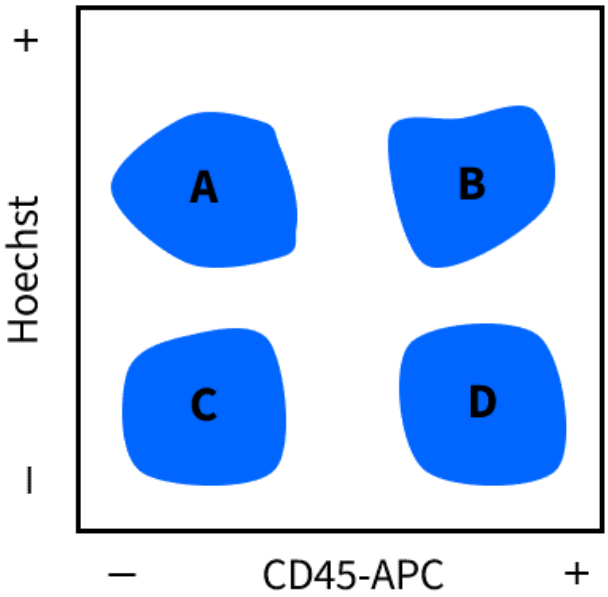


Figure 3: Flow cytometry results, testing for Hoechst and CD45.

Four clusters (A, B, C, D) are therefore possible due to the four possible combinations of Hoechst-/+ and CD45-/+, as shown in Figure 2.

Q1. Which clusters would each of the cell populations (iRBC, uRBC, uRTIC, WBC) fall into? Note that it is possible for different cell populations to be represented in the same cluster, and not all clusters may be represented by a cell population. **(40 points)**
(*Enter the correct letter to the correct row.*)

Cell Population	**Cluster**
iRBC	
uRBC	
uRTIC	
WBC	

After analysis, you obtain these numbers:

Cell Type	**Population Size/millions**
iRBC	0.3
uRBC	8.9
uRTIC	0.8
WBC	8.0

Q2. Calculate the parasitaemia of the patient based on the above data. **(20 points)**
(Enter your answer as a percentage correct to 3 s.f. Do not include the percent (%) sign.)

When flow cytometry is unavailable, parasitaemia can also be estimated by doing a thin blood smear and viewing it under a microscope. It is assumed that the distribution of iRBC throughout the slide is uniform. The iRBC and total mature RBC from several microscope fields are counted. For this question, let us just use one microscope field.

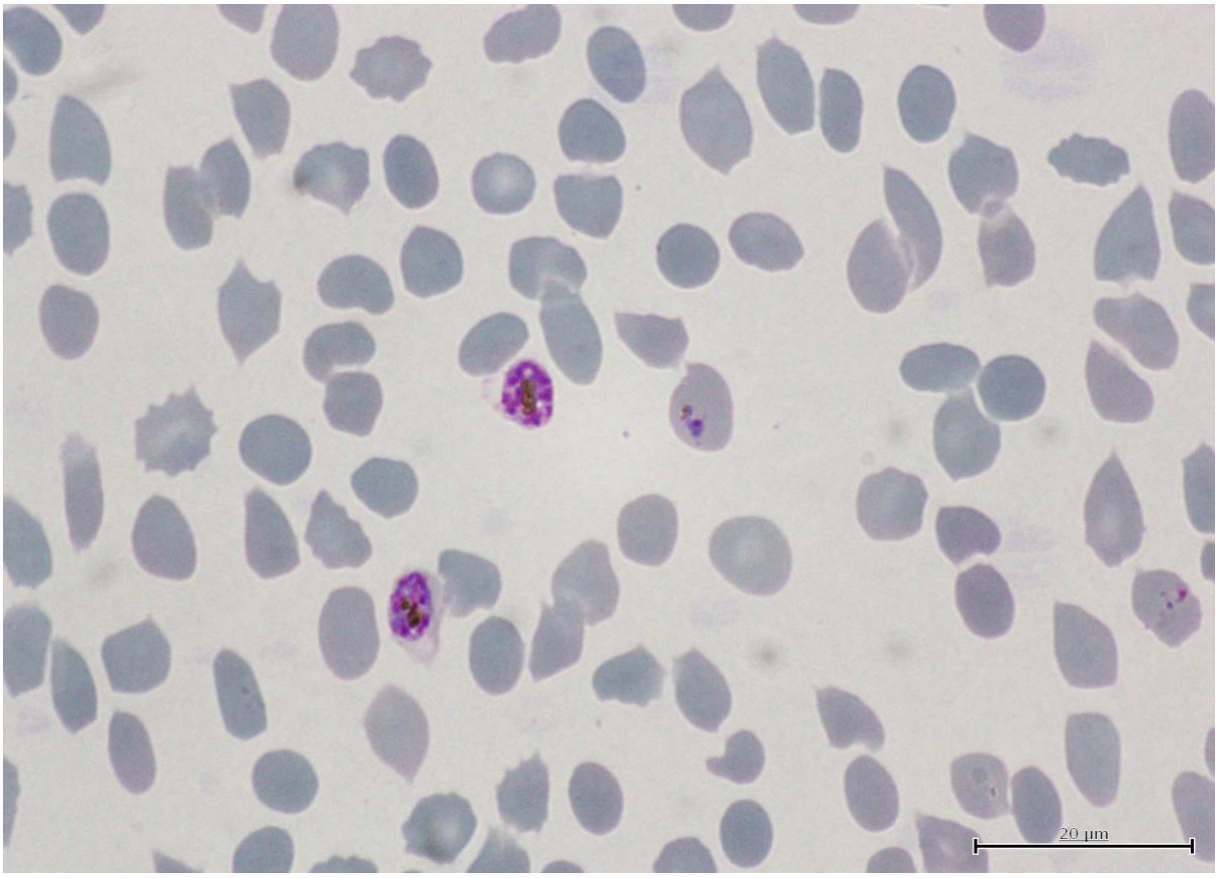


Figure 4: Thin blood smear of a *P. falciparum* culture, one microscope field.
Parasites appear purple and the red blood cells appear grey.

Q3. Calculate the parasitaemia based on the microscope field in Figure 4. For RBCs that are on the edge of the field and partially out of the field, count the ones on the left-hand side and top side of the field, but exclude the ones on the right-hand and bottom sides of the field. **(30 points)**
(Enter your answer as a percentage correct to 3 s.f. Do not include the percent (%) sign.)

Plasmodium parasites convert excess haem, which is toxic to the parasite, to haemozoin which is stored in a vacuole. Magnetic Activated Cell Sorting (MACS) is a method used in many *Plasmodium* studies. A parasite culture containing infected RBCs is passed through a column of magnetic beads.

Q4. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. Gating is unable to exclude data from several cells passing through the laser beam simultaneously.
B. Debris is identified through its low FSC values.
C. uRBC is present in the MACS eluent.
D. iRBC is retained in the MACS column.

Q5. What is the purpose of MACS? **(20 points)**
(Select all correct options.)

A. To enrich and isolate the iRBC
B. To increase rate of infection so more uRBC becomes iRBC for analysis
C. To induce eddy currents to elute the haemozoin
D. To magnetise the *Plasmodium* parasites for extraction from iRBC
E. To separate total RBC from WBC
F. To measure haem content in RBC

Answers and Explanations

Q1.

Answer: **A, C, C, B**

Explanation:

A. Infected red blood cells will contain *Plasmodium* DNA, hence they will be Hoechst-positive. Red blood cells are non-nucleated haematopoietic cells, hence they will be CD45-negative. So, the answer is **A**.
B. Uninfected red blood cells will not contain any DNA, hence they will be Hoechst-negative. Red blood cells are non-nucleated haematopoietic cells, hence they will be CD45-negative. So, the answer is **C**.
C. Uninfected reticulocytes will not contain any DNA, hence they will be Hoechst-negative. Reticulocytes are non-nucleated haematopoietic cells, hence they will be CD45-negative. So, the answer is **C**.
D. White blood cells contain a nucleus that contains DNA, hence they will be Hoechst-positive. White blood cells are nucleated haematopoietic cell, hence they will be CD45-positive. So, the answer is **B**.

Q2.

Answer: **3.26**

Explanation: The formula to calculate parasitaemia is given in the question.

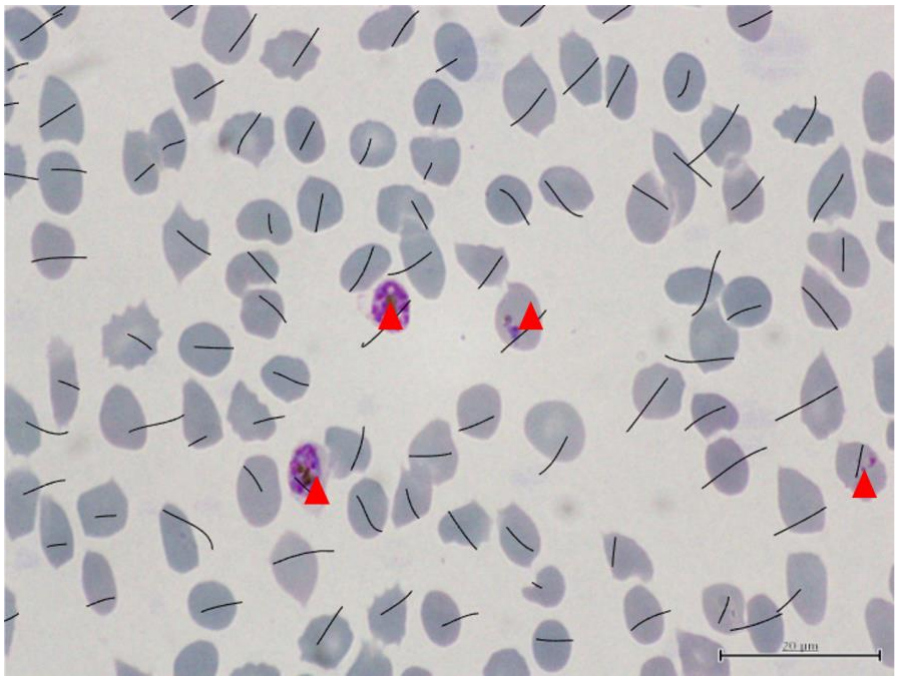
Total mature RBC includes iRBC and uRBC and excludes uRTIC as reticulocytes are immature. Hence, the calculation is as follows:

$$\left(\frac{0.3}{0.3+8.9}\right) \times 100\% = 3.26\ \%$$

Q3.

Answer: **4.26 or 4.30 or 4.21**

Explanation: We can count the number of iRBC and RBC below:



The red triangle indicates the iRBC which can be identified by the purple colouration.

Hence:

$$Number\ of\ iRBC\ =\ 4$$

$$Number\ of\ total\ RBC\ =\ 94$$

$$Parasitaemia\ =\ \left(\frac{4}{94}\right) \times 100\%\ =\ 4.26\%$$

4.30 and **4.21** were also accepted as participants may have miscounted the total RBC count as 93 or 95 respectively.

Note: Participants were provided with a link to an editable image of Figure 4 during the contest.

Q4.

Answer: **FTTT**

Explanation:

A. Gating is able to exclude data from several cells passing through the laser beam simultaneously. When several cells pass through the laser beam simultaneously, the FSC value will be multiple of the typical FSC value for one singular cell, while the SSC value remains the same. Hence, gating is able to exclude data from doublet, triplet, multiplet cells through the multiplet FSC values.
B. Debris is far smaller in size as compared to cells and will have low FSC values. It can thus be identified through its low FSC values.
C. There is no magnetic material in uRBC, hence they will not be retained in the MACS column and will be passed out into the eluent.
D. iRBC contains haemozoin which is produced by the *Plasmodium* parasite and is iron-containing and therefore magnetic. Hence, it will be attracted to the magnetic MACS column and it will be retained in the MACS column.

Q5.

Answer: **A**

A. iRBC is retained in the column and uRBC is passed out in the eluent. Hence, when extracting what is retained on the column, it will be concentrated with iRBC only.
B. Magnetising the culture does not increase the rate of infection.
C. Haemozoin-containing red blood cells will be retained in the column, not eluted.
D. You cannot “magnetise” the parasites, which simply contain magnetic haemozoin. “Magnetising” the parasites will also not aid in its extraction.
E. uRBC is not retained in the column along with iRBC, and will be eluted out along with WBC.
F. MACS is not a quantitative technique.

Credits

Figure 1: Florentin, A., Stephens, D. R., Brooks, C. F., Baptista, R. P., & Muralidharan, V. (2020). Plastid biogenesis in malaria parasites requires the interactions and catalytic activity of the Clp proteolytic system. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(24), 13719–13729. https://doi.org/10.1073/pnas.1919501117

Figure 2: *Introduction to flow cytometry*. abcam. (n.d.). https://www.abcam.com/protocols/introduction-to-flow-cytometry

P17: This problem is a bully

(170 points)

Figure 1 shows a section of Plant A.

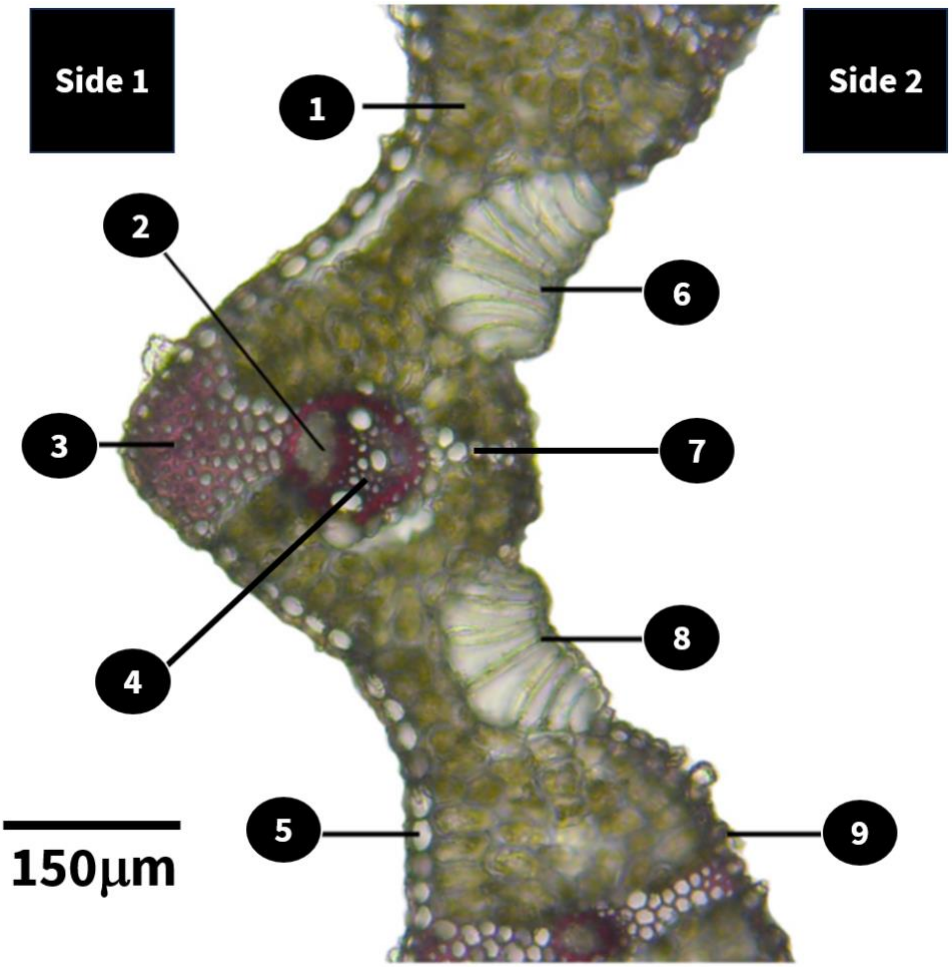


Figure 1: Plant A section.

Q1. Indicate which plant organ Figure 1 belongs to. **(10 points)**
(Enter the correct word or phrase. Do not pluralise.)

Q2. Indicate which of the following statements are true or false. **(50 points)**
(Mark each statement as true or false.)

A. Side 1 represents the abaxial side.
B. Figure 1 represents a mesophyte.
C. Figure 1 is a longitudinal section.
D. The stem of Plant A likely has no pith.
E. Structure 7 is likely along the midrib.

Q3. Match the numbers of the structures (1-9) in Figure 1 to the following descriptions. If there is no such structure or process, type None. If there is more than one answer, type in all possible answers in numerical order without space or punctuations in between. **(40 points)**
(Enter the correct numbers to the correct rows. If there is more than one possible number, enter all correct numbers in numerical order.)

Description	**Number**
Presence of lignin	
Chlorenchyma	
Trichomes	
Transports mineral salts	

Q4. Indicate whether of the following statements regarding the plant in Figure 1 are true or false. **(50 points)**
(Mark each statement as true or false.)

A. Stratified columnar mesophyll is present.
B. There are high levels of photorespiration at high temperatures such as 35°C.
C. There are high activity levels of PEP carboxylase in bundle-sheath cells.
D. RuBisCO is more complementary in conformation and charge to carbon dioxide in this plant than in most plants.
E. Optimum temperature for photosynthesis is 30 to 40°C.

Q5. Which of the following most accurately represents the approximate area of the phloem vascular bundles in Figure 1? **(20 points)**
(Select the correct option.)

A. 0.000955 mm^2
B. 0.00172 mm^2
C. 0.00612 mm^2
D. 0.0188 mm^2
E. 0.187 mm^2
F. 1.91 mm^2
G. 194 μm^2
H. 1210 μm^2
I. 11 700 μm^2
J. 1.00 m^2

Answers and Explanations

Q1.

Answer: **Leaf**

Explanation: It is trivial as the general shape gives away that it is a leaf. The striate arrangement of the vascular bundles as well as large amounts of chlorophyll appearing as green on the cross section suggests that it is a leaf.

Q2.

Answer: **TFFTT**

Explanation:

A. The bulliform cells, labelled 8, are found on the adaxial (upper) side of the leaf, thus side 1 is the abaxial (lower) side and side 2 is the adaxial (upper) side.
B. The presence of bulliform cells suggest that it is likely a xerophyte with the presence of bulliform cells to allow the leaf to curl up to reduce water loss by transpiration.
C. Figure 1 is a transverse section.
D. The striate venation pattern as evidenced by the vascular bundles running parallel to each other suggests that this is a monocot plant. Monocot stems have no true pith and instead have ground tissue.
E. As this is a transverse section, structure 7 likely is running along the midrib as it is superior to the vascular bundle which is most likely the most pronounced vein as it is larger and thicker. Moreover, the bend in the shape of the leaf implies that it is likely the midrib.

Q3.

Answer: **34, 1, None, 4**

Explanation: The nine structures from 1-9 are as follows: chlorenchyma, phloem, sclerenchyma, xylem, epidermal cell, bulliform cell, parenchyma cell, bulliform cell, cuticle.

A. Both structures 3 and 4 appear red in Figure 1 due to the presence of lignin stained red by phloroglucinol-HCl.
B. Structure 1 appears as green as it contains a lot of chlorophyll.
C. There are no trichomes. Structure 9 is not a trichome as trichomes would likely be longer and more prominently shaped. The small protrusions on structure 9 are likely due to the tearing of the cuticle during sample preparation.
D. This is clearly the xylem as the mineral salts are dissolved in water.

Q4.

Answer: **FFFFT**

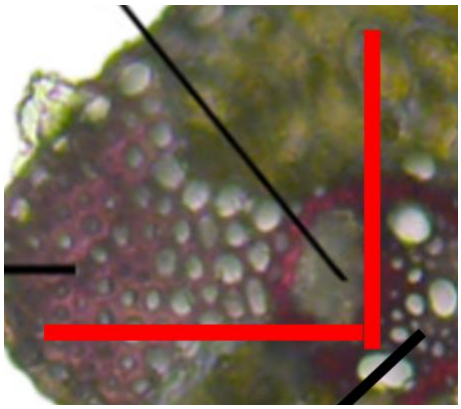
Explanation: The presence of Kranz anatomy suggests that the plant is a C4 plant.

A. The mesophyll is radiated due to the presence of Kranz anatomy.
B. There are low rates of photorespiration as the spatial separation of photosynthesis in C4 plants help to minimise photorespiration by reducing the interaction of oxygen with RuBisCO.
C. PEP carboxylase acts in the mesophyll cells.
D. The RuBisCO enzyme is still the same in conformation and charge in this plant. The difference is in the spatial separation, with RuBisCO acting in the bundle-sheath cells.
E. As this is a C4 plant, it is adapted to photosynthesis at higher temperatures.

Q5.

Answer: **B**

Explanation: The length of the phloem bundle (Structure 2) is approximately $70\mu m$ while the width is approximately $25\mu m$. The diagram below shows the $150\mu m$ line beside the phloem bundle in Figure 1.



Thus, the area is:

$$Area = 70\mu m \times 25\mu m = \mathbf{1750\mu m^2} = 1750mm^2 \times 10^{-3} \times 10^{-3} = \mathbf{0.00175mm^2}$$

Hence the answer is B. Option F is incorrect as it forgets to multiply by 10^{-3} a second time as the micrometer prefix is converted to the millimeter prefix twice.

Credits

Figure 1: Modified from Mader, A., Langer, M., Knippers, J., & Speck, O. (2020). Learning from plant movements triggered by bulliform cells: The Biomimetic Cellular Actuator. *Journal of The Royal Society Interface*, *17*(169), 20200358. https://doi.org/10.1098/rsif.2020.0358

P18: Training in the Avidya Forest

(190 points)

Population genetics is a field of biology that studies the distribution of allele frequencies within and between populations of organisms. Rather than looking at the cellular level (in the case of molecular genetics) or individual level (in the case of classical genetics), population genetics focuses on the bigger picture — how the overall distribution of traits changes within collections of individuals.

Inheritance is random in nature. For example, the allele for a particular gene in a diploid organism is passed down from parent to offspring in a 50-50-coin flip. However, through a macroscopic lens, these random events provide data for statistical inferences, which can then be applied to make predictions about the overall nature of a particular genetic trait.

In this problem, you will be sent to the Avidya Forest to learn population genetics from a trained researcher, Tighnari. Tighnari will guide you through two lectures before putting your skills to the test in a case study.

Tighnari's Lecture, Chapter 1: Hardy-Weinberg equilibrium

Figure 1: Godfrey Harold Hardy (1877-1947), a statistician who studied population genetics

The **Hardy-Weinberg equilibrium** is a fundamental principle in the study of population genetics. A population is said to be in Hardy-Weinberg equilibrium if there are no evolutionary influences acting upon the population, such as natural selection or genetic drift. When a population is in Hardy-Weinberg equilibrium, both the **allele** and **genotype frequencies remain constant** from generation to generation.

Allele and genotype frequencies, being frequencies, are independent of population size. Hence, changes in population size are not an evolutionary influence and do not violate the Hardy-Weinberg principle.

In reality, no population is in perfect Hardy-Weinberg equilibrium. However, we can still use this principle to make inferences when a population is *near* Hardy-Weinberg equilibrium.

Tutorial, Chapter 1

A population of creatures, the Spinocrocodile, have three different eye colours: white, red, and pink. Eye colour is controlled by one gene with two alleles (*R*/*r*) that are incompletely dominant.

- Genotype *RR*: Red eye colour
- Genotype *Rr*: Pink eye colour
- Genotype *rr*: White eye colour

Tighnari had commissioned an ecologist to study the population of the Spinocrocodile. However, when he received the report, part of the report was damaged by water and the ink smudged.

Eye colour	Red	Pink	White	Total
Number	???	???	144	400

Q1. Assuming the Spinocrocodile population is and had been in Hardy-Weinberg equilibrium for at least the past generation, calculate the number of Spinocrocodiles in the population that have red eyes and pink eyes respectively. **(20 points)**
(Enter your answers correct to the nearest whole number.)

Colour of Eyes	Number
Red	
Pink	

Tighnari's Lecture, Chapter 2: Chi-squared test

A statistical tool we can use to determine if observed data is statistically different from our calculated expectations is the **chi-squared test**.

$$\chi^2 = \sum \left[\frac{(o-e)^2}{e} \right]$$

The **chi-squared value** can be calculated as the sum of $\frac{(o-e)^2}{e}$ for each phenotype, where o and e are the observed data and expected data respectively. For example, if the number of white-fur mice observed is 40 when it was expected to be 50, $\frac{(o-e)^2}{e} = \frac{(40-50)^2}{50} = 2$.

Depending on the degrees of freedom, the chi-squared value can be converted to the **probability** (also known as the P-value) that one obtains data as extreme as your data. Hence, this indicates the probability that the discrepancy in the data is due to chance. In research, a small probability indicates a statistically significant result. In biology, most researchers use a critical P-value of 0.05.

Tutorial, Chapter 2

A population of creatures, the Rishboland Tiger, has three sideburn lengths: long, dwarf, and no sideburn. The gene controlling sideburn length has two alleles with incomplete dominance, where the no sideburn allele *N* is dominant to the long sideburn allele *n*.

In 2002, Tighnari collected data on the number of Rishboland Tigers with each sideburn length. The population of Rishboland tigers were in Hardy-Weinberg equilibrium. 20 years later, he collected data again on the same population of Rishboland Tigers to investigate whether the population is still in Hardy-Weinberg equilibrium.

Sideburn Length	**Long**	**Dwarf**	**No sideburn**
Number (2002)	3	54	243
Number (2022)	15	101	584

A chi-squared test was done to determine whether the population of Rishboland Tigers remained in Hardy-Weinberg equilibrium from 2002 to 2022. Several possible hypotheses are listed below.

A. There are **no** significant differences between the phenotypic frequencies in the observed and expected data. Hence, the population **did not remain** in Hardy-Weinberg equilibrium from 2002 to 2022.
B. There are **no** significant differences between the phenotypic frequencies in the observed and expected data. Hence, the population **remained** in Hardy-Weinberg equilibrium from 2002 to 2022.
C. There are significant differences between the phenotypic frequencies in the observed and expected data. Hence, the population **did not remain** in Hardy-Weinberg equilibrium from 2002 to 2022.
D. There are significant differences between the phenotypic frequencies in the observed and expected data. Hence, the population **remained** in Hardy-Weinberg equilibrium from 2002 to 2022.

Q2. Match the appropriate null and alternative hypotheses from the hypotheses (A-D) above. **(20 points)**

(Match the correct letter to the correct row.)

Hypothesis	**Option (A-D)**
Null hypothesis	
Alternative hypothesis	

Q3. State the chi-squared value. **(30 points)**

(Enter your answer correct to 3 s.f.)

Q4. The test was carried out with a significance level of 0.05. Indicate whether the following statements are true or false. **(40 points)**

(Mark each statement as true or false.)

A. The null hypothesis should be rejected in favour of the alternative hypothesis.
B. The population of Rishboland Tigers had evolved.
C. The results of the chi-squared test show that natural selection selects against heterozygous genotypes for sideburn length in the population of Rishboland Tigers.
D. The results could be explained by a sexual preference for partners with similar sideburn lengths in the population of Rishboland Tigers.

Case Study: Eleazar

Good job sitting through the past two lectures! Tighnari believes that you are ready to assist him with his research. He tells you that the nation of Sumeru (which you are both in right now) had a major problem: there exists an incurable disease called Eleazar, which causes hard scales to form on the skin.

Tighnari had been researching this disease for many years, and he vowed to continue until, in his words, “Eleazar and all its notorious effects have been rid from this world.”

He slides over a stack of paper, and you see a pedigree on the front page (Figure 2). “The first case, many centuries ago,” he says indiscriminately, without a hint of expression on his face. “Unlucky fellow.”

Individual I-1 was recorded to be the **first case of Eleazar** in the country. Since then, the country had been on lockdown, so no one could enter in or out.

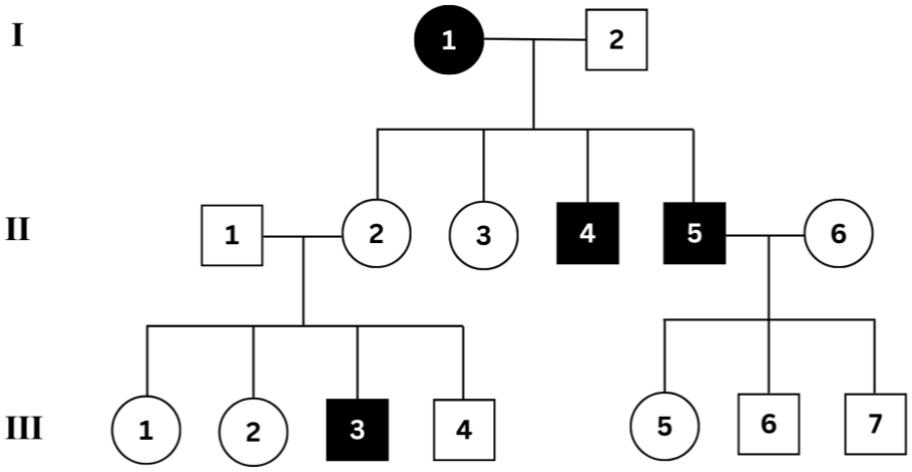


Figure 2: Pedigree

You flip the page and the table of data in Table 1. In the year 2024, Tighnari collected data from a sample of 1,840 people from Sumeru.

Table 1: Data from 1840 people in Sumeru

	Females	Males	Total
Afflicted with Eleazar	72	236	308
Total	920	920	1840

“Find anything?” Tighnari asks. You look at the table carefully, and something seems off. “Hold on a minute,” you say, “isn’t this a disproportionate fraction of males who are affected? That’s so unfair”

“Well, some variations here and there are expected when we collect data in reality, no?” he retorts.

You adamantly believe that that is not the case. “Seems like something more,” you reply, “but I’m not sure how to prove it.”

You decide to perform a chi-squared analysis to determine whether the number of males and females with Eleazar in the table significantly differs from the expected numbers should the disease affect both sexes equally. Your calculated results are seen in the table below.

My First Eleazar chi-squared test

	Observed	Expected	$\frac{(o-e)^2}{e}$
Females with Eleazar	72	**a**	**f**
Females without Eleazar	848	**b**	**g**
Males with Eleazar	236	**c**	**h**
Males without Eleazar	684	**d**	**j**
		Total:	104.881

Q5. The test was carried out with a significance level of 0.05. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The critical chi-squared value is 9.488.
B. The probability that the discrepancy between the observed data and expected data is not due to random chance alone is extremely high.
C. Value **a**, value **b**, value **c** and value **d** are equal.
D. Value **f** and value **h** are equal.

Tighnari seems impressed with your work. However, his smile has all but vanished.

“Right, back to business,” he says, walking over to you, “because I have a report to send to *my* mentor. He’s asked me to extrapolate the nation’s current predicament and approximate the number of Eleazar cases twenty years from now.”

“What? Why?” You seem confused.

“Well, he’s working on some… treatment for Eleazar and wants to know how much time he has before the situation gets out of hand.” Tighnari’s face is grim.

“Isn’t it out of hand already?” But perhaps both of you already know the answer.

In any case, Tighnari has entrusted you with the final part of his report. Use the knowledge that he has passed to you prior and research well before submitting the calculations back to him.

Q6. With reference to Figure 2 and Table 1, calculate the proportion of females in the tribe that are heterozygous for Eleazar in the year 2044, assuming the population is in Hardy-Weinberg equilibrium, and that the relative fitness of individuals with Eleazar is 1. **(40 points)**
(Enter your answer correct to 3 s.f.)

You slide your report to Tighnari. He takes a peek at your messy calculations, but seems content with it.

“Thank you, traveller. Now, we wait for next year.”

Answers and Explanations

Q1.

Answer: **64, 192**

Explanation: Let p and q denote the allele frequencies for *R* and *r* respectively.

$$q^2 = \frac{144}{400} = 0.36$$

$$q = 0.6$$

$$p = 1 - 0.6 = 0.4$$

$$\text{Proportion of red-eyed Spinocrocodiles} = p^2 = 0.16$$

$$\text{Number of red-eyed Spinocrocodiles} = 0.16 \times 400 = 64$$

$$\text{Proportion of pink-eyed Spinocrocodiles} = 2pq = 0.48$$

$$\text{Number of red-eyed Spinocrocodiles} = 0.48 \times 400 = 192$$

Q2.

Answer: **B, C**

Explanation: Options A and D cannot be true because a significant difference between the phenotypic frequencies in the observed and expected data indicates that the population is no longer in Hardy-Weinberg equilibrium, and vice versa.

The null hypothesis states the lack of a difference, while the alternative hypothesis states that there *is* a difference.

Q3.

Answer: **14.6**

Explanation: If the population of Rishboland Tigers had always been in Hardy-Weinberg Equilibrium, the allele frequencies should remain constant from 2002 to 2022. There are 300 Rishboland Tigers in 2002.

$$p = \frac{243 \times 2 + 54 \times 1}{600} = 0.9$$

$$q = 1 - 0.9 = 0.1$$

There are 700 Rishboland Tigers in 2022.

	Observed	**Expected**	$\frac{(o-e)^2}{e}$
Long	15	$0.1^2 \times 700 = 7$	$9\frac{1}{7}$
Dwarf	101	$2 \times 0.1 \times 0.9 = 126$	$4\frac{121}{126}$
No sideburn	584	$0.9^2 \times 700 = 567$	$\frac{289}{567}$
		Sum:	14.6 (3 s.f.)

Q4.

Answer: **TTFT**

Explanation:

A. Since the chi-squared value is greater than the critical chi-squared value, the null hypothesis is rejected.
B. The population has evolved because the population is not in Hardy-Weinberg equilibrium. In other words, the allele frequencies are shifting.
C. The results of the chi-squared test cannot "show" any conclusion other than rejecting the null hypothesis, or not.
D. Results can be explained independent of the test. Sexual preference is a condition that breaks the Hardy-Weinberg equilibrium.

Q5.

Answer: **FTTT**

Explanation:

A. The critical chi-squared value is 7.815 as the degrees of freedom is 3. You get the value 9.488 when the degrees of freedom is 4.
B. The null hypothesis is rejected in favour of the alternate hypothesis as the calculated chi-squared value is greater than the critical chi-squared value.
C. Because proportions must be less than one, the chi-squared value will be much smaller. Note that this will become the chi-squared test for homogeneity of proportions.
D. Both values f and h are the same (43.7).

Q6.

Answer: **0.396**

Explanation:

The pedigree indicates that Eleazar is a sex-linked recessive disease.

We cannot assume that the allele frequency between females and males are the same.

$$q_F = \sqrt{\frac{72}{920}} \approx 0.27975$$

$$q_M = \frac{236}{920} \approx 0.25652$$

$$q_{E\ (equilibrium)} = \frac{2}{3} \times 0.27975 + \frac{1}{3} \times 0.25652 \approx 0.272$$

We weight the values as such because females have two X chromosomes while males have one X chromosome, and there are the same number of females and males.

We can assume the population reaches an equilibrium (characterised by $q_F = q_M = q_E$) in 10,000 years.

Hence, number of heterozygous females $= 2p_E q_E = 2 \times 0.272 \times (1 - 0.272) = 0.396$

P19: Malicious Malicia's Malaria Magnum Opus II

(140 points)

Malaria is caused by a protozoan parasite belonging to the *Plasmodium* genus. The genus *Plasmodium* belongs to the phylum Apicomplexa. Nearly all apicomplexans possess the apicoplast, a special type of organelle. Figure 1 shows a *P. falciparum* parasite infecting a red blood cell.

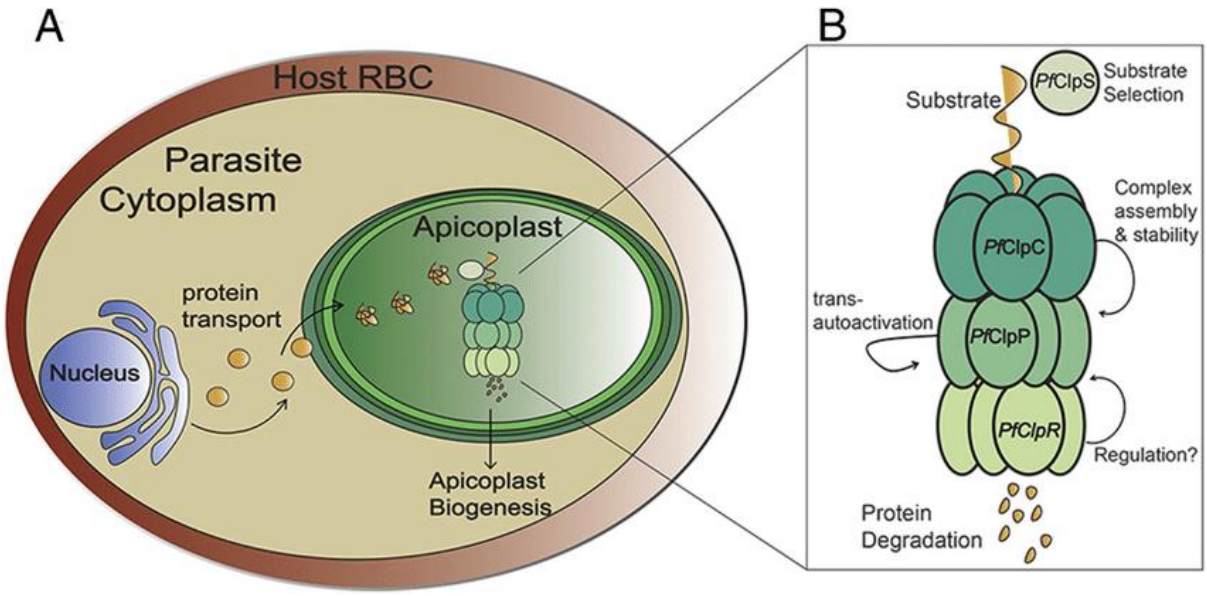


Figure 1: Diagrammatic representation of the *P. falciparum* parasite.

Q1. In terms of origin, which organelle is the apicoplast most similar to? **(10 points)**
(Select the correct option.)

A. Ribosome
B. Chloroplast from land plants
C. Chloroplast from brown algae
D. Mitochondria
E. Amyloplast
F. Lysosome

Q2. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. When the apicoplast is isolated, processed and run through gel electrophoresis, bands will appear when stained with ethidium bromide.
B. The Clp complex in Figure 1 is only involved in lipolysis.
C. *Plasmodium falciparum* is stained using the Ziehl-Neelsen acid-fast stain.
D. More than one parasite can exist in an erythrocyte.

Figure 2 shows the life cycle of the parasite.

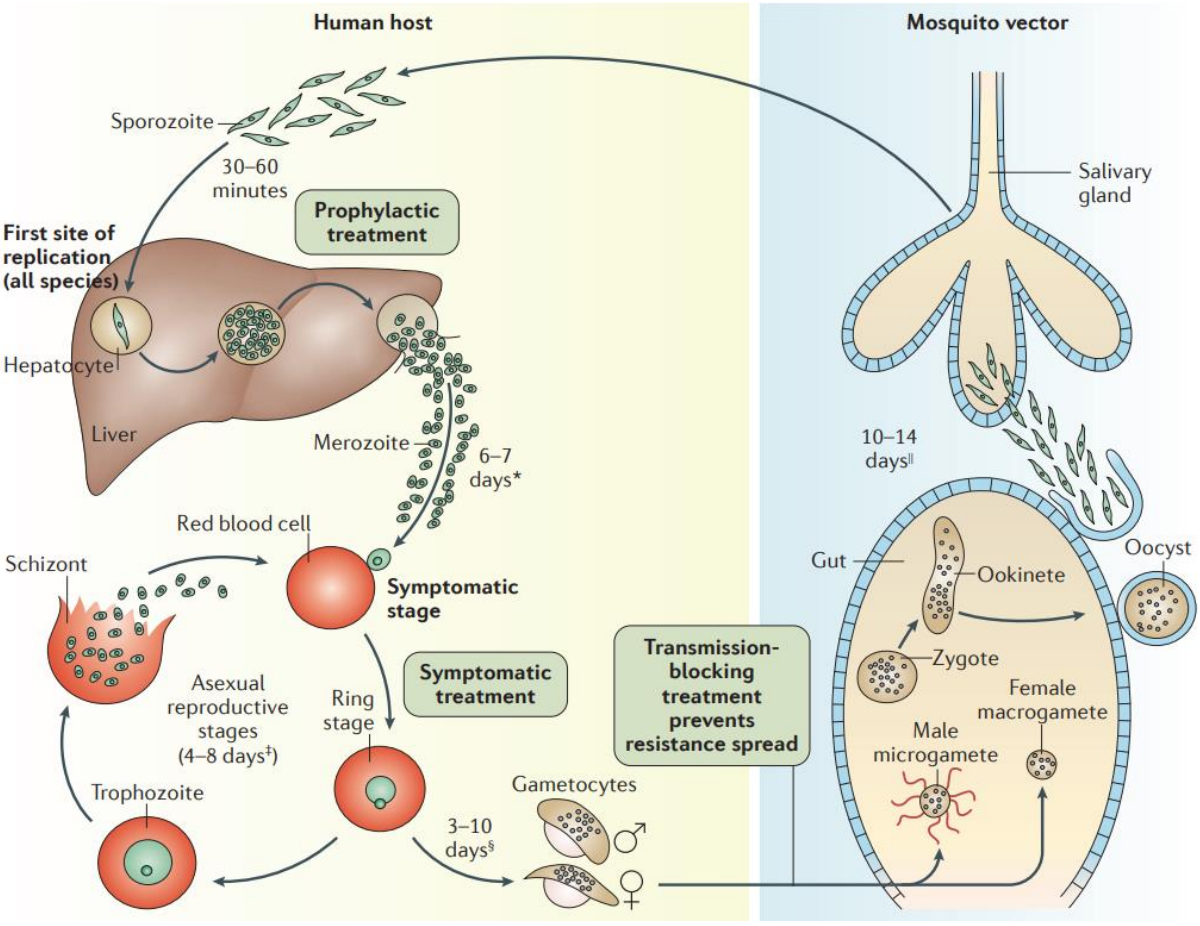


Figure 2: Diagram of the *P. falciparum* lifecycle.

Q3. The apicoplast is essential to the survival of the Plasmodium parasite. One of the functions of the apicoplast is fatty acid synthesis. Select all the options that represent likely outcomes of disrupting this function of the apicoplast. **(20 points)**

(Select the correct options.)

A. The parasite will die immediately.
B. The parasite is no longer able to invade the host red blood cells.
C. The parasite will not be able to form merozoites at the liver stage.
D. The parasite will no longer be able to be transmitted from host to vector.

Q4. Which parasite development stages would you expect to be able to see in an *in vitro* culture with red blood cells? **(30 points)**

(Select the correct options.)

A. Sporozoite
B. Merozoite
C. Ring stage
D. Trophozoite
E. Schizont
F. Gametocyte
G. Zygote
H. Ookinete

Four life stages of *P. falciparum* as seen under the microscope are labelled Images W-Z in Figure 3.

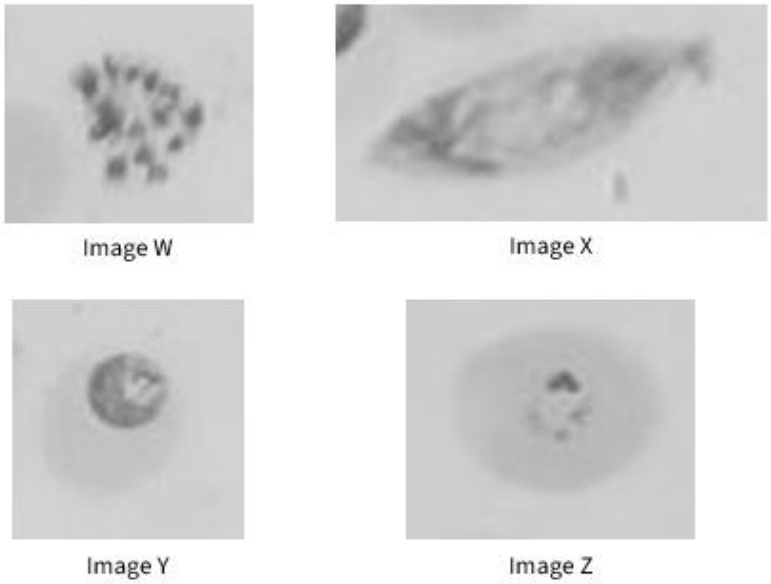


Figure 3: Life stages of *P. falciparum*

Q5. Match the images (W-Z) to their respective life stages. Use Figure 2 to aid you. **(40 points)**

(*Enter the correct answer to each row.*)

Image	**Life Stage**
W	
X	
Y	
Z	

Answers and Explanations

Q1.

Answer: **C**

Explanation: The question stem “In terms of origin” is intended to direct participants to the fact that the apicoplast arose from secondary endosymbiosis. Hence, the answer should be an organelle that also arose from secondary endosymbiosis. Of all these organelles, only chloroplast from brown algae originated from secondary endosymbiosis.

Q2.

Answer: **TFFT**

Explanation:

A. The apicoplast contains DNA, which will show up as bands under gel electrophoresis.
B. The apicoplast is also involved in several other functions, such as protein degradation (as shown in Figure 1).
C. The Ziehl-Neelsen acid-fast stain is used to stain certain bacteria, but *Plasmodium* is not a bacteria.
D. This is true as evidenced by microscopic examination of infected red blood cells.

Q3.

Answer: **B, C**

A. Disrupting fatty acid synthesis is not immediately lethal.
B. The invasion of red blood cells requires passing through the phospholipid cell membrane of red blood cells, which gives us a hint that fatty acids are involved. The process of invasion is complicated, requiring the parasite to release several macromolecules that induce structural changes in the cell membrane of red blood cells necessary for invasion. One of these macromolecules is lipids.
C. To form the individual phospholipid cell membranes for each merozoite, fatty acid synthesis is essential to produce enough phospholipids.
D. Host-to-vector transmission does not involve movement through phospholipid cell membranes and is simply the process of an *Anopheles* mosquito drinking blood that contains infected red blood cells.

Q4.

Answer: **B, C, D, E**

Explanation:

A. Sporozoite - not found in blood culture as this stage is only found during vector-to-host transmission.
B. Merozoite
C. Ring stage - found in asexual blood culture
D. Trophozoite - found in asexual blood culture
E. Schizont - found in asexual blood culture
F. Gametocyte - only found in blood culture that has begun sexual differentiation
G. Zygote - not found in blood culture as this stage is only found in the mosquito midgut
H. Ookinete - not found in blood culture as this stage is only found in the mosquito midgut

Q6.

Answer: **Schizont, Gametocyte, Trophozoite, Ring (stage)**

Explanation:

A. W - a schizont appears as a cluster of several purple dots
B. X - the distinctive banana shape tells us it is a stage V gametocyte
C. Y - through the process of elimination, this is a trophozoite. Alternatively, it is identifiable through the presence of a brown speck in amid the purple area, which is the haemozoin (tends to be more apparent in the trophozoite stage).
D. Z - as per its name, the ring stage looks like a ring bearing a jewel

Credits

Figure 1: Florentin, A., Stephens, D. R., Brooks, C. F., Baptista, R. P., & Muralidharan, V. (2020). Plastid biogenesis in malaria parasites requires the interactions and catalytic activity of the Clp proteolytic system. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(24), 13719–13729. https://doi.org/10.1073/pnas.1919501117

Figure 2: Phillips, M. A., Burrows, J. N., Manyando, C., van Huijsduijnen, R. H., Van Voorhis, W. C., & Wells, T. N. (2017). Malaria. *Nature Reviews Disease Primers*, *3*(1). https://doi.org/10.1038/nrdp.2017.50

P20: Daddy Long Legs

(130 points)

Many metabolic reactions in the body involve condensation polymerisation, including DNA and polypeptides. In polymerisation reactions, two monomers are joined together with the removal of a small molecule (Figure 1). Thus, the mass of the polymer is less than the total mass of both the monomers due to the loss of the molecule.

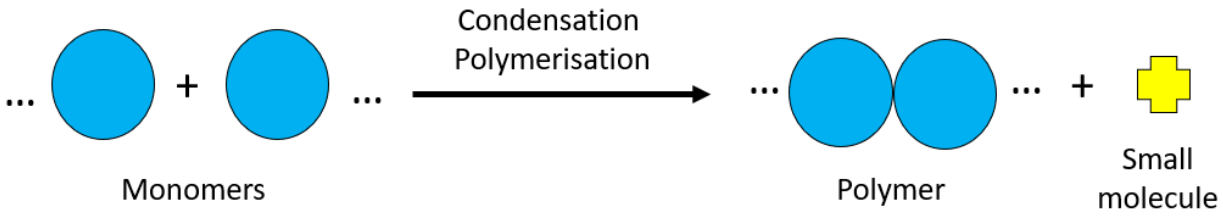


Figure 1: Condensation Polymerisation

Q1. Indicate which of the following biological processes are descriptions of **condensation polymerisation**. **(30 points)**
(Select all correct options.)

A. Formation of tRNA in the nucleus
B. Complementary base pairing between DNA strands to form a DNA double helix
C. Water forming a continuous stream in the xylem for transpirational pull
D. Formation of cellulose by cellulase
E. Formation of lipids by joining of fatty acids and glycerol
F. Formation of amylopectin in plant cells
G. Aminoacyl-tRNA synthetase joining amino acid to tRNA molecule
H. Formation of keratin in hair
I. Blood coagulation by platelets and plasma proteins
J. Binding of insulin to the insulin receptor
K. Methylation of DNA

Luke is already familiar with polymerisation in proteins, and is thus curious to understand more about polymerisation in nucleic acids. He is curious about the changes to the DNA helix structure when the nucleobases are changed.

Luke modified several nucleobases found in nucleic acids and produced deoxysulfuric dihydronine triphosphate (dSTP), deoxyyttrbiumine triphosphate (dYTP), deoxyjeromine triphosphate (dJTP), and deoxylimomethyline triphosphate (dLTP). These four molecules are the deoxyribonucleoside triphosphates (dNTP) of S, Y, J and L respectively. L is similar to cytosine as both can be methylated by methyltransferase. S (pyrimidine) base pairs with Y (purine) and J (pyrimidine) with L (purine). These

dNTP molecules are known to polymerise in the same manner as dATP, dCTP, dGTP, and dTTP to form nucleic acids.

Q2. How many different DNA triplets (forming mRNA codons) can be produced by these 4 modified nucleobases (S, Y, J, L) and the four canonical nucleobases (A, T, G, C), assuming that at least one nucleobase must be either S, Y, or J? **(20 points)**
(Enter your answer correct to the nearest whole number.)

Luke used these modified dNTPs to construct three different ssDNA strands. However, he does not know the identity of each dNTP molecule and thus labelled them A, B, C, and D. Their sequences and their mass can be seen below. The masses of the dNTP molecules and other relevant molecules are given below as well.

DNA strand	Sequence	Mass/g mol^{-1}
1	ABDDCADBBABB	4221
2	CABADABABDD	3833
3	ABBACADABBAA	4205

Molecule	Mass/g mol^{-1}
Methyl Group	15
Acetyl Group	43
Phosphate	95
Pyrophosphate	174
dSTP	490
dYTP	498
dJTP	521
dLTP	530

Q3. Identify dNTP molecules A, B, C, and D. **(40 points)**

(Match the correct letter to the correct row.)

dNTP	**Label (A, B, C, D)**
dSTP	
dYTP	
dJTP	
dLTP	

Q4. Indicate whether the following statements are true or false. **(40 points)**

(Mark each statement as true or false.)

A. These DNA strands cannot be transcribed due to the lack of a start codon (AUG).
B. If the ssDNA strands are base paired with their complementary strand forming a dsDNA molecule, their mass will be doubled.
C. Methylation of limomethyline (L) in DNA strand 3 will cause a theoretical rise in mass of 56g for each mole of the DNA strand.
D. If Luke creates a ssDNA strand where the percentage of J is 35%, the percentage of L will be 15%.

Answers and Explanations

Q1.

Answer: **A, F, H**

Explanation:

A. tRNA is formed in the nucleus by transcription of the tRNA genes. Transcription involves polymerisation of the nucleotides.
B. Complementary base pairing is not a form of polymerisation condensation as a small molecule is not lost and it is simply temporary hydrogen bonding between two bases.
C. Water forms a continuous stream due to hydrogen bonding between the electropositive hydrogen atom and electronegative oxygen atom in water. This causes cohesion and adhesion, which is not polymerisation condensation.
D. Cellulose is digested by cellulase and not formed by it.
E. Formation of lipids is not condensation despite the loss of a water molecule as there is no repeated linking of small monomers to form a polymer.
F. Amylopectin is formed by condensation of α-glucose monomers to form starch with the loss of a water molecule. Amylopectin differs from amylose due to the presence of $\alpha(1,6)$ branching.
G. The joining of the amino acid to the 3'-CCA stem of tRNA is not polymerisation condensation as there is no repeated linking of monomers.
H. Keratin is a protein formed by the condensation of amino acids.
I. Blood coagulation occurs because platelets and the plasma proteins pile up together and clump up to form an insoluble fibrin; this is not a direct result of polymerisation.
J. Insulin binds to its receptor as they are complementary in conformation and charge. This binding is also temporary and not a permanent covalent bond.
K. DNA methylation only occurs once to each cytosine residue.

Q2.

Answer: **387**

Explanation: We first find out how many triplets can be formed using the 8 nucleobases. Since the nucleobase can be repeated, $\mathbf{8^3}$ is the number of triplets as there are 8 choices for each nucleotide in the triplet.

Since at least one nucleobase must be S, Y, or J, we need to exclude cases where they are not in the triplet. The number of triplets where S, Y, or J are not in the triplet is the same as the number of triplets where only A, T, G, C, and L are used. Hence the number of triplets is $\mathbf{5^3}$.

Therefore, the required number of triplets is:

$$\boldsymbol{Required\ number\ of\ triplets = 8^3 - 5^3 = 387}$$

Q3.

Answer: **D, A, C, B**

Explanation: A DNA strand is formed by the condensation of dNTPs with the loss of a pyrophosphate molecule for each condensation reaction. For $\boldsymbol{n}$ bases, there are $\boldsymbol{n-1}$ condensation reactions, as every base will polymerise with that on the right except for the last base. Thus, with each condensation reaction, 174g mol^{-1} will be lost as pyrophosphate.

Hence, the mass of a DNA strand can be calculated as:

$$\boldsymbol{Mass = Mass\ of\ all\ dNTPs - (n-1)(174)}$$

First, let A, B, C and D represent the molar mass of their respective dNTPs. We then count the number of each dNTP in each DNA strand:

	A	B	C	D	$\boldsymbol{n}$	$\boldsymbol{(n-1)(174)}$
Strand 1	3	5	1	3	12	1914
Strand 2	4	3	1	3	11	1740
Strand 3	6	4	1	1	12	1914

We can thus set up three simultaneous equations:

$$3A + 5B + C + 3D - 1914 = 4221 \Rightarrow 3A + 5B + C + 3D = 6135$$

$$4A + 3B + C + 3D - 1740 = 3833 \Rightarrow 4A + 3B + C + 3D = 5573$$

$$6A + 4B + C + D - 1914 = 4205 \Rightarrow 6A + 4B + C + D = 6119$$

With four variables, we know we need at least four simultaneous equations. Since we know A, B, C, D must correspond to S, Y, J, L in some order, we can construct another equation using all four dNTPs:

$$A + B + C + D = 490 + 498 + 521 + 530 = 2039$$

There is no need to subtract any pyrophosphate molecule here because A, B, C and D represent the masses of the dNTPs.

Solving the four simultaneous equations with a calculator or MATLAB (or by hand), we get:

$$A = 498; B = 530; C = 521; D = 490$$

Hence by comparing the masses, we can find out the identity of each dNTP molecule.

Q4.

Answer: **FFTF**

Explanation:

A. The start codon is only needed in translation. It is the presence of the TFIID recognition element (TATA box) (-25) in the promoter as well as the upstream TFIIB recognition element (-35) that initiates transcription.
B. This is in general not true as each nucleotide binds to the complementary base not the same base.
C. There are four limomethyline residues in DNA strand 3 that can be methylated. The addition of four methyl groups will cause a rise of $4(15 - 1) = 64g\ mol^{-1}$ because one hydrogen atom is lost for each addition of the methyl group. It is also well known that the molar mass of a hydrogen atom is 1g mol^{-1}.
D. The DNA strand is single stranded so there is no relationship between the proportions of each nucleotide.

P21: Trihybrid Trouble

(190 points)

A plant has three genes that influence the intensity of the colour of its petals. Let these genes have alleles *A/a*, *B/b* and *C/c*. They have an additive effect as such:

- *AA*, *BB* or *CC* contribute 4 units of pigment each
- *Aa*, *Bb* or *Cc* contribute 2 units of pigment each
- *aa*, *bb* or *cc* contribute 1 unit of pigment each

Hence, a plant with genotype *AabbCc* would have petals with 5 units of pigment in them whereas another flower with genotype *AABBcc* would have 9 units of pigment in its petals. To get the ball rolling and the flowers blooming, we first cross two flowers with genotype *AaBbCc*. *(Hint: You can use Excel to create three Punnett squares, each measuring the effect of each of the three genes, and then add them up to get the final distribution.)*

Q1. Which genotype-phenotype relation matches the above scenario the best? **(10 points)**
(Select the correct option.)

A. Complete dominance
B. Incomplete dominance
C. Codominance
D. Overdominance
E. Underdominance

Q2. Indicate the number the units of pigment in the flower petals of plants with the following genotypes. **(30 points)**
(Enter a whole number to each row. Do not include any units.)

Genotype	**Amount of pigment/units**
AaBBCC	
Aabbcc	
aabbcc	

Q3. How many possible genotypes would result from the cross? **(10 points)**
(Enter a whole number.)

Q4. Which of the following units of pigment would be modal? **(30 points)**
(Select all correct options.)

A. 1
B. 2
C. 3
D. 4
E. 5
F. 6
G. 7
H. 8
I. 9
J. 10
K. 11
L. 12

Q5. If we keep picking flowers from the offspring of the cross, what would be the average pigment value? The value need *not* be a whole number. **(30 points)**
(Enter your answer correct to 3 s.f.)

Suddenly, the plant's pollinators go near blind and cannot see any flowers whose petals have 9 units of pigment or fewer. You should assume they have an equal chance of landing on any flower they can see and will pollinate said flower.

Q6. What is the probability of the pollinator landing on a flower with 12 units of pigment in its petals? **(10 points)**
(Enter your answer as a decimal correct to 3 s.f.)

Q7. Which of the following plant genotypes have flowers that the pollinator can see and hence land on? **(10 points)**
(Select all correct options.)

A. Triple homozygous dominant
B. Triple heterozygous
C. Triple homozygous recessive

Wanting to study the pigment more, we extract it from the petals and make a solution. We put the solution into the spectrophotometer in cuvettes of 1-cm width and collect the following data:

Genotype	**Absorbance/a.u.**
AABBCC	0.891
AABbCC	0.750
aaBbCc	0.364
aabbcc	0.222

Q8. If we want to model the above values in a graph of absorbance against the number of pigment units with a best-fit straight line, what would its gradient be? **Enter your answer correct to 2 s.f. (20 points)**
(*Enter your answer correct to **2 s.f.**)*

Where necessary, use the **2 s.f. values of the gradient and y-intercept** of the equation of the graph you obtained in **Q8** to solve the following questions.

The Beer-Lambert Law is used in spectrophotometry to determine the concentration of a light-absorbent species. The formula is seen below:

$$A = \varepsilon c l$$

Where A refers to absorbance, ε is the molar extinction coefficient ($M^{-1} cm^{-1}$), c is the concentration of the species (M), and l is the length of solution that the light passes through the cuvette (cm).

Q9. The solution obtained from the plant with genotype *aabbcc* is found to have a concentration of 0.08M. Assuming that we are using cuvettes of 1 cm width, use the Beer-Lambert Law to find the molar extinction coefficient in $M^{-1} cm^{-1}$. **(20 points)**
(Enter your answer correct to 3 s.f.)

Q10. A careless student over-dilutes the pigment solution extracted from the flower petals of a plant with genotype *aaBBCc*. He obtains an absorbance value of 0.174. How many times was the solution diluted? **(10 points)**

(Select the correct option.)

A. 10x
B. 8x
C. 5x
D. 4x
E. 3x
F. 2x

Q11. What would the expected absorbance value be from the solution extracted from a flower belonging to a plant with genotype *AAbbCC*? **(10 points)**

(Enter your answer correct to 3 s.f.)

Answers and Explanations

Q1.

Answer: **B**

Explanation:

A. The heterozygous phenotype is not the same as that of homozygous dominant, thus it is not complete dominance.
B. The heterozygous phenotype trait value is between homozygous dominant and homozygous recessive, thus it is incomplete dominance.
C. Both recessive and dominant phenotypes are not expressed simultaneously, thus it is not codominance.
D. No information is given about the fitness of the different phenotypes.
E. No information is given about the fitness of the different phenotypes.

Q2.

Answer: **10, 4, 3**

Explanation:

A. For AaBBCC: 2 + 4 + 4 = 10
B. For Aabbcc: 2 + 1 + 1 = 4
C. For aabbcc: 1 + 1 + 1 = 3

Q3.

Answer: **27**

Explanation: For each gene, three genotypes are possible - homozygous dominant, heterozygous and homozygous recessive. In our case of a trihybrid cross, there are $3^3 = 27$ possible genotypes.

Q4.

Answer: **E, G, H**

Explanation: If you used the hint, you would get something like the bottom-right table!

FOR A	ABC	ABc	AbC	aBC	Abc	aBc	abC	abc
ABC	4	4	4	2	4	2	2	2
ABc	4	4	4	2	4	2	2	2
AbC	4	4	4	2	4	2	2	2
aBC	2	2	2	1	2	1	1	1
Abc	4	4	4	2	4	2	2	2
aBc	2	2	2	1	2	1	1	1
abC	2	2	2	1	2	1	1	1
abc	2	2	2	1	2	1	1	1

FOR B	ABC	ABc	AbC	aBC	Abc	aBc	abC	abc
ABC	4	4	2	4	2	4	2	2
ABc	4	4	2	4	2	4	2	2
AbC	2	2	1	2	1	2	1	1
aBC	4	4	2	4	2	4	2	2
Abc	2	2	1	2	1	2	1	1
aBc	4	4	2	4	2	4	2	2
abC	2	2	1	2	1	2	1	1
abc	2	2	1	2	1	2	1	1

FOR C	ABC	ABc	AbC	aBC	Abc	aBc	abC	abc
ABC	4	2	4	4	2	2	4	2
ABc	2	1	2	2	1	1	2	1
AbC	4	2	4	4	2	2	4	2
aBC	4	2	4	4	2	2	4	2
Abc	2	1	2	2	1	1	2	1
aBc	2	1	2	2	1	1	2	1
abC	4	2	4	4	2	2	4	2
abc	2	1	2	2	1	1	2	1

TOTAL	ABC	ABc	AbC	aBC	Abc	aBc	abC	abc
ABC	12	10	10	10	8	8	8	6
ABc	10	9	8	8	7	7	6	5
AbC	10	8	9	8	7	6	7	5
aBC	10	8	8	9	6	7	7	5
Abc	8	7	7	6	6	5	5	4
aBc	8	7	6	7	5	6	5	4
abC	8	6	7	7	5	5	6	4
abc	6	5	5	5	4	4	4	3

pigment count	3	4	5	6	7	8	9	10	11	12
no	1	6	12	11	12	12	3	6	0	1

An alternative method would be to calculate, by hand, the probability of each combination of genotypes that give rise to each pigment unit. We calculate the number of possible ways to obtain phenotype by looking at the genotype combination, and then divide by 64 possible ways (8^2).

Number of Units	Combination(s)	Number of Ways
1	Not possible	0
2	Not possible	0
3	1+1+1	$1 \times 1 \times 1 = 1$
4	1+1+2	$1 \times 1 \times 2 \times \frac{3!}{2!\,1!} = 6$
5	1+2+2	$1 \times 2 \times 2 \times \frac{3!}{2!\,1!} = 12$
6	1+1+4 2+2+2	$1 \times 1 \times 1 \times \frac{3!}{2!\,1!} + 2 \times 2 \times 2 = 11$
7	1+2+4	$1 \times 2 \times 1 \times \frac{3!}{1!\,1!\,1!} = 12$
8	2+2+4	$2 \times 2 \times 1 \times \frac{3!}{2!\,1!} = 12$
9	1+4+4	$1 \times 1 \times 1 \times \frac{3!}{2!\,1!} = 3$
10	2+4+4	$2 \times 1 \times 1 \times \frac{3!}{2!\,1!} = 6$
11	Not possible	0
12	4+4+4	$1 \times 1 \times 1 \times \frac{3!}{3!} = 1$

Q5.

Answer: **6.75**

Explanation: Refer to the picture above. You can think of this as a weighted average: $\frac{432}{64} = 6.75$.

Alternatively, we can use the table above:

$$Weighted\ average = \frac{3 + 6 \times 4 + 12 \times 5 + 11 \times 6 + 12 \times 7 + 12 \times 8 + 3 \times 9 + 6 \times 10 + 12}{64} = 6.75$$

Q6.

Answer: **0.143**

Explanation: Refer to the picture or the table. Only focussing on those flowers with at least 10 units of pigment, we get:

$$\frac{1}{6\ +\ 0\ +\ 1} = 0.143$$

Q7.

Answer: **A**

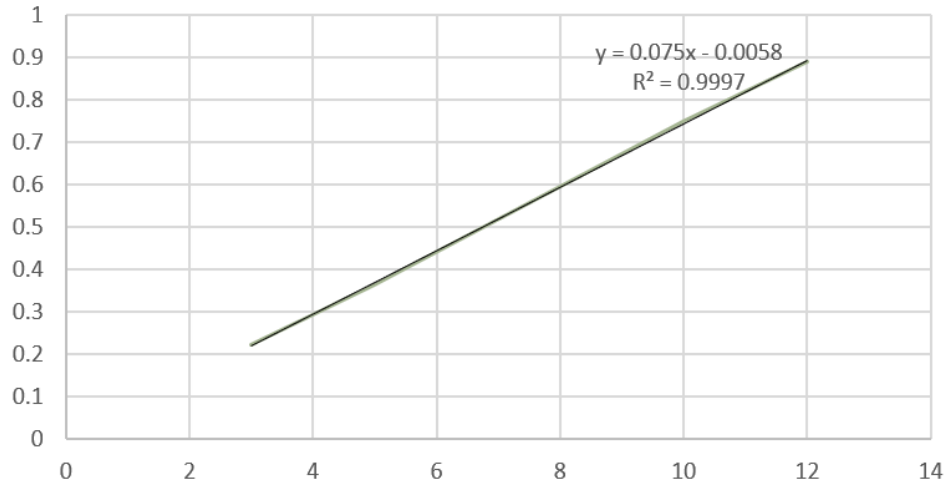
Explanation:

A. Triple homozygous dominant flowers would have $3 \times 4 = 12 > 9$ units of pigment.
B. Triple heterozygous would have flowers $3 \times 2 = 6 < 9$ units of pigment.
C. Triple homozygous recessive would have flowers $3 \times 1 = 3 < 9$ units of pigment.

Q8.

Answer: **0.075 or 0.074**

Explanation: By plotting the graph using the points in the table in Microsoft Excel, MATLAB, Desmos, or any other graphing software, you will get the equation of the best-fit line as seen below:



Although the question has specifically requested for only the values in the table of the graph to be modelled, and for the best-fit line to be drawn, it is understandable that some participants may decide that the point (0, 0) should be included in the points as it is known that there is 0 absorbance with no pigments. The same answer of **0.075** will still be obtained.

Alternatively, you may model the graph as $y = mx$ as the graph should theoretically pass through the origin. Using this method, you will obtain **0.074** as the answer. Hence, either answer was accepted.

Specifically, the equation of the line using the three methods respectively are:

$$y = 0.075x - 0.0058$$

$$y = 0.075x - 0.0025$$

$$y = 0.074x$$

Q9.

Answer: **2.78**

Explanation: From the Beer-Lambert law, $A = \varepsilon cl$, so $\varepsilon = \frac{A}{cl} = \frac{0.222}{0.08 \times 1} = 2.775 = 2.78$

Q10.

Answer: **E**

Explanation: Based on the graph, for a pigment value of 1 + 4 + 2 = 7 units, the absorbance value would have been $0.075 \times 7 - 0.0058 = 0.519$ (or $0.075 \times 7 - 0.0025 = 0.523$ or $0.074 \times 7 = 0.518$). These numbers are around triple that of the actual value obtained, so we can deduce that a 3x dilution had happened.

Q11.

Answer: **0.667 or 0.669 or 0.673 or 0.666**

Explanation: There would be 9 units of pigment. Either you calculate this based on **Q9** and scale the concentration value based on 3 units of pigment having an absorbance of 0.08M:

$$A = 2.775 \times \left(\frac{9}{3}\right) \times 0.08 \times 1 = 0.667$$

or with the graph from **Q8**:

$$A = 0.075 \times 9 - 0.0058 = 0.669$$

OR:

$$A = 0.075 \times 9 - 0.0025 = 0.673$$

OR:

$$A = 0.074 \times 9 = 0.666$$

P22: Bend it like Bee-ckham

(230 points)

From your education in the Regnia Symposium, you have learnt about how scout honeybees perform a waggle dance when they find a location with nectar and pollen or a new nest site. The dance conveys information regarding the distance and direction of the location. An explanation of the waggle dance is given in Figure 1, so as to refresh your memory.

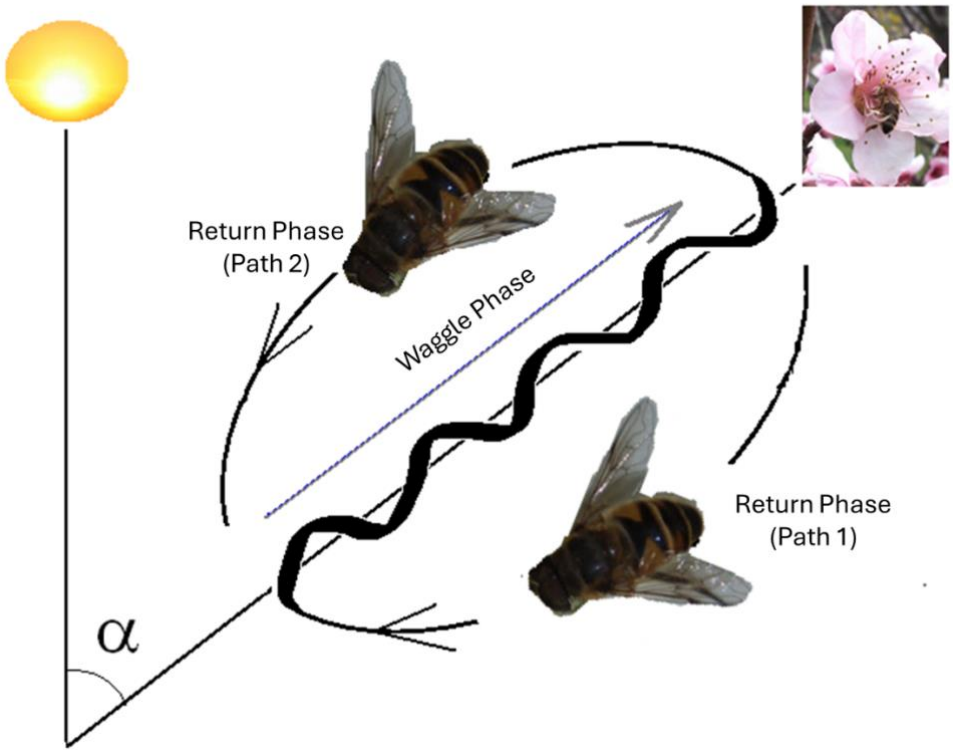


Figure 1. A diagram of the waggle dance performed by honeybees

The dance can be divided into two parts: the waggle phase and the return phase. The waggle phase is the actual part of the dance that encodes information about the location. Firstly, the dance is done on the vertical plane of the honeycomb, with upwards being the sun's position at that time of the day. The angle of the waggle phase denotes the relative angle of the location with respect to the sun, represented in Figure 1 by α. Secondly, the duration of the waggle phase encodes the distance. It is generally agreed that 0.1s of waggle duration correlates with around 100 metres in distance.

Now, you have been called to the planet Arrakis by St. Alia of the Knife, a psychic with a soft spot for zoology, to study some of the native animals and characterise them. On the spaceship from your home planet to Arrakis, you were given the following information about the first animal you have to study, the Flutters:

To try and get away from the Harkonnen soldiers, the Shai-Hulud worms on the planet have evolved the ability to fly. They are colloquially referred to as the Flutters by the Imperial Planetologists governed by House Atreides. There are two species (both capable of flight) and each has its own extant population, one in the North Frontier and the other in the South Frontier. Both populations have only one Queen, whereas all the other Flutters and their offspring can be Scouts. The ancestral population was split into two roughly genetically similar groups (the North Frontier population and the South Frontier population) after the Lord of the Earth struck upon Arrakis and erected a chain of Pillars using the secret Otherworldly Spell of ***Dominus Lapidus****. Over time, the two populations have ended up as two distinct species.*

After this separation, only the Flutters of the North Frontier evolved the distinctive Fluttwerk dance. This dance is a spectacle, usually performed by a Scout Flutter in front of the Queen Flutter. The energetic dance frequently summons sandstorms, so the Fremen cry out the phrase ***fardana itha*** *(which means 'duststorm comes' in their native tongue) just before the Scout Flutter commences the Fluttwerk.*

The Flutters, like their ancestors, are fiercely territorial. They can sense the presence of foreigners in their land and frequently attack them in retaliation. This means spice harvesters and rogue Harkonnen soldiers (who escaped the Holy War waged by Paul Atreides upon the alliance of Houses Harkonnen and Corrino) regularly incur the wrath of these otherwise docile animals. However, their own is lost in the process. Provided are some resources gathered by the native Fremen during their spice expeditions that can offer more information about the Flutters.

Population	Habitat area/km^2	Population size	Number of predators or enemies	Number of prey	Water availability/m^3 water
Ancestral population	545,600	8246	12,504	45,014	400,000
North Frontier population	326,500	4935	23,965	26,940	239,370
South Frontier population	219,100	3311	4,895	18,074	160,630

You are assured that the number of predators or enemies, number of prey and water availability has remained fairly constant throughout time and the values given are the mean of all annual measurements taken for the population ever since their existence. The population size of the ancestral population was measured at its peak, right before the split. The population size of the North Frontier and South Frontier populations was taken right after the split.

Q1. Which of the following is the most likely evolutionary pressure behind the evolution of the Fluttwerk behaviour in the North Frontier population? **(20 points)**
(Select the correct option.)

A. Decreased per capita water availability (m^3/no. of individuals)
B. Increased predator or enemy density (no. of predators or enemies/km^2)
C. Decreased prey density (*units redacted*)
D. Increased prey density (*units redacted*)
E. Increased intra-population competition for space (*units redacted*)
F. Increased intra-population competition for prey (no. of prey/no. of individuals)
G. Decreased water availability (m^3/km^2)

Several possible graphs for the number of mortalities per 1000 individuals per week over 4500 years are given in Figure 2.

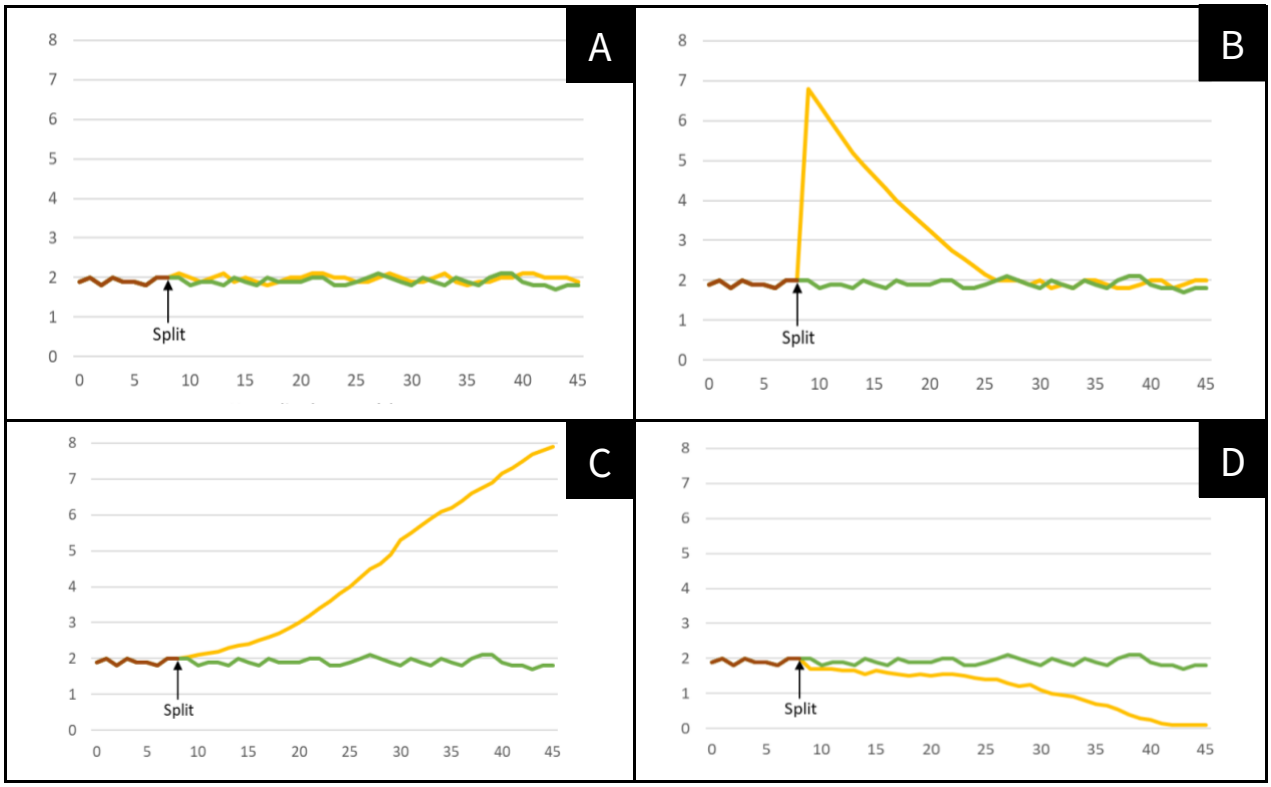


Figure 2: Possible graphs of number of mortalities per 1000 individuals per week over 4500 years. y-axis represents number of deaths per week per 1000 individuals. x-axis represents the year in thousands. Yellow line: North Frontier, Green line: South Frontier, Brown line: Ancestral population. The point of splitting of the ancestral population is indicated with an arrow.

Q2. Based on Figure 2, what is the most plausible graph for the annual number of mortalities in the population? **(10 points)**

(Select the correct option.)

A. A
B. B
C. C
D. D

Q3. Which type of speciation mechanism fits that of the Flutters the best? **(10 points)**

(Select the correct option.)

A. Allopatric Speciation
B. Parapatric Speciation
C. Peripatric Speciation
D. Sympatric Speciation

Q4. Match the species concepts to the *possible* observations you can make of the Flutters. **(40 points)**

(Match the correct number to the correct row.)

1. Biological
2. Ecological
3. Morphological
4. Phylogenetic

Observation	**Species Concept (1-4)**
The two species prey on different organisms and inhabit geographically separate areas.	
Only the North Frontier species has evolved the Fluttwerk, which makes it an apomorphy.	
The two species produce infertile offspring when cross-reproducing.	
The North Frontier species have a different pattern on their scales and wings as compared to the South Frontier species.	

After reading all the information provided and consolidating your understanding, your superior tasks you to understand the Fluttwerk. The characteristics of the Fluttwerk that can be varied are the duration of the dance, which way the abdomen of the Scout Flutter points during the dance and which wing is fluttered.

Many brilliant graduates of the Regnia Symposium have tried and failed at this task, so you set your mind to be the first one to crack this secret the Flutters hold. However, the Fremen used to revere the Shai-Hulud and hence hold their descendant Flutters in high regard. As a result, you are strictly forbidden from keeping them captive and using them as experimental subjects. To understand the Fluttwerk better, you only have data of past fights that the Fremen happened to notice. They are recorded in the Arrakis Annals found at the Imperial Library and your transcribed set of notes are below. Will this unlikely occurrence and the honeybee's waggle dance offer any clues? Only time will tell. Go forth and maybe, just maybe, the Regnia Symposium will laud you in the centuries to come.

Year of Fight	**Individual Location**	**Fluttwerk Characteristic**		
		Duration of Fluttwerk	**Abdomen Pointing**	**Wing(s) fluttered**
9922 AG*	4 spice harvesters due north, 200m away	30 seconds	Down	Right
9961 AG	10 spice harvesters due northeast, 100m away	30 seconds	Down	Right
10130 AG	2 Harkonnen soldiers due east, 150m away	10 seconds	Left	Left
10137 AG	1 Harkonnen soldier and 2 spice harvesters due southeast, 200m away	15 seconds	Left	Both
10147 AG	4 spice harvesters and 2 Harkonnen soldiers due south, 100m away	30 seconds	Down	Both
10148 AG	1 Harkonnen soldier and 1 spice harvester due southwest, 200m away	10 seconds	Right	Both
10152 AG	1 spice harvester due west, 50m away	5 seconds	Right	Right

10166 AG	5 Harkonnen soldiers and 3 spice harvesters due northwest, 150m away	30 seconds	Down	Both
10177 AG	3 Harkonnen soldiers due north, 50m away	15 seconds	Down	Left
10180 AG	5 spice harvesters due south, 100m away	30 seconds	Down	Right

**AG refers to After Guild, with 0 AG being the year when the Spacing Guild successfully monopolised space travel. Before House Harkonnen took over Dune in 10130 AG, only spice harvesters were recorded as unfortunate victims of the Flutters.*

Q5. What do the Fluttwerk features represent? **(40 points)**
(Match the correct number to the correct row.)

1. Duration of Fluttwerk
2. Direction the abdomen points in
3. Which wings are fluttered
4. Not coded for

Information regarding threat	**Fluttwerk feature (1-4)**
Type of intruder	
Number of intruders	
Distance of intruders	
Direction of intruders	

Your superior checks your hypothesis against all the recorded fights, and surprisingly it seems to hold up! Extremely well, in fact. You smile inwardly, proud of what you have achieved. The days seem to fly by incredibly fast… you now proceed to study the different plants around the desert and marvel at their adaptations in such an arid climate. One day, a Fremen group runs towards the camp your colleagues have set up. They're mumbling and gesturing wildly while behind you, there seems to be dust flying everywhere. And amidst this chaos you hear the words you thought you wouldn't in this lifetime, '**fardhana itha**'. Slowly, you piece together from what the Fremen tell you that 7 spice harvesters of Muad-dib's have entered the North Frontier from the southwest and were detected by the Scout Flutter 150m away. Your supervisor is too shocked to react but you, as a true science graduate of the Regnia Symposia, immediately get your sand-resistant and self-cooling writing pad out.

Q6. What would the Fluttwerk in the above case look like? **(30 points)**
(Match the correct number to the correct description.)

1. 5s
2. 10s
3. 15s
4. 20s
5. 25s
6. 30s
7. 35s
8. Abdomen pointing down
9. Abdomen pointing left
10. Abdomen pointing right
11. Abdomen pointing up
12. Only left-wing fluttering
13. Only right-wing fluttering
14. Both wings fluttering
15. No wings fluttering

Fluttwerk feature	**Option (1-15)**
Duration of Fluttwerk	
Direction the abdomen points in	
Which wings are fluttered	

As all proper ethologists do, you have a lingering desire to investigate the proper basis of the Fluttwerk with Tinbergen's four questions. You have already found out the function (purpose) of and causation (stimulus) leading to the Fluttwerk, and sadly are inadequately equipped to examine the evolution of the behaviour over the 38,000 years since the chain of Pillars was erected. That leaves you with the fourth question, that of ontology - **how did the Fluttwerk develop within a North Frontier Flutter's lifespan?**

Conveniently, you find a young Flutter from the North Frontier who has sadly been separated from its group. You sneakily put a convincing model of a spice harvester near it and watch it from the distance. The poor thing gets close to the model and eats it up in one gulp. It gets scratched on the head by the metal though, and it makes this weird star-shaped scar. What's more interesting than that is that it surprisingly does not perform the Fluttwerk. A few days later, it reunites with the group (woohoo)! You don't see how you can further your research on the behaviour's ontology, so you wrap this funky study up and head back to rest for the evening.

***

Time passes. It's been a few decades since you came to Arrakis. Now, it's time for you to pack up and return to your home planet for retirement. You decide to say goodbye to the Flutters that defined your career on this desert planet in a symbolic way to celebrate your success. The decoding of the Fluttwerk got you the Gesserit Medal from the Sisterhood of Science, after all. On your way there, you see a Flutter (as big as they get) with a star-shaped scar… and then you see it once more.

In 10221 AG, the last-ever Fluttwerk you will get to study.

You are suddenly consumed by your thoughts over the work you did with the Flutters over the past few decades, but there is little time to mull. You need to pack for the Spacing Guild ship. You run back to your shelter, emotions running high and your mind in a whirl. You suppose this is the end.

> **Q7**. Reflecting on your work, indicate whether the following statements are true or false. **(40 points)**
> *(Mark each statement as true or false.)*
>
> A. The North Frontier population underwent the founder effect.
> B. This two-species situation can be described as cladogenesis.
> C. The chain of Pillars can be thought of as the isolating mechanism.
> D. The Fluttwerk is a learned behaviour.

Answers and Explanations

Q1.

Answer: **B**

Explanation: After calculating each option, it is clear that only in option B has the predator/enemy density increased for the North population after the divide, hence this is a possible evolutionary pressure behind the Fluttwerk behaviour. As for the other options, this remains approximately the same for both populations before and after the divide. Hence, those are unlikely driving forces for the evolution of the Fluttwerk behaviour.

Q2.

Answer: **B**

Explanation: A divide causing a rapid spike in predator/enemy density will cause a rapid spike in the number of deaths per week per 1000 individuals, followed by a gradual decline back to the initial baseline over several thousands of years as the Fluttwerk behaviour evolves and becomes more common in the North population. Whereas for the South population, the predator/enemy density has remained the same so the number of deaths per week per 1000 individuals should remain fairly constant for this population after the divide. The only graph that fits this description is option B.

Q3.

Answer: **A**

Explanation:

A. A physical, geographical barrier between two populations that prevents them from reproducing with each other, eventually causing speciation
B. A population is spread out over a very wide geographical range, so even though there is no physical barrier to prevent mating, individuals tend to mate within a smaller geographical range and are thus speciated according to differences in the same environment.
C. A physical barrier also prevents mating, but one resultant population is much smaller and the other, and this smaller population will tend to have more unique traits due to the smaller population size.
D. No geographical barrier between two populations, but occupying different ecological niches causes them to separate over time.

Q4.

Answer: **2, 4, 1, 3**

Explanation:

A. Using the ecological species concept, you may conclude that they are two different species because they occupy different ecological niches.
B. Using the Phylogenetic species concept, you may conclude that they are two different species because they have genetically diverged (assuming the Fluttwerk behaviour has a genetic basis).
C. Using the Biological species concept, you may conclude that they are two different species because they cannot produce viable offspring.
D. Using the Morphological species concept, you may conclude that they are two different species because their morphologies are different.

Note: There was an error during the contest where the options on the interface did not match those in the question. This question was therefore voided.

Q5.

Answer: **3, 1, 4, 2**

Explanation: If you reduce the data given to its bare form, you will get something like this:

Total Number	Spice Harvester	Harkonnen	Direction	Distance	Duration of Fluttwerk	Abdomen Pointing	Wing(s) fluttered
4	4		N	200	30 seconds	Down	Right
10	10		NE	100	30 seconds	Down	Right
2		2	E	150	10 seconds	Left	Left
3	2	1	SE	200	15 seconds	Left	Both
6	4	2	S	100	30 seconds	Down	Both
2	1	1	SW	200	10 seconds	Right	Both
1	1		W	50	5 seconds	Right	Right
8	3	5	NW	150	30 seconds	Down	Both
3		3	N	50	15 seconds	Down	Left
5	5		S	100	30 seconds	Down	Right

Then, it's a matter of deduction. One strategy possible would be to associate the quantitative variables together (i.e., number of enemies or their distance with the Fluttwerk duration) and see if it works out. The other way possible would be to filter sort every column in the above table to see if there is a correlation. If there is, then trends would be observed in another column that was not filter sorted. You can try this out with the total number of enemies and see the Fluttwerk duration scale accordingly. The direction is the hardest part, and it is based on 3 arcs marked in red below. The black lines are the 8 cardinal directions, with N being at the 12 o' clock mark.

For the top arc (encompassing NW, N and NE), the abdomen points downwards. For the bottom left arc (encompassing W and SW), the abdomen points right. For the last arc (encompassing E and SE), the abdomen points left. As for S which is between the two lower arcs, the abdomen still points down.

Q6.

Answer: **6, 10, 13**

Explanation: Similar to the previous question, with your knowledge of the Fluttwerk, you now need to encode the details of the enemies into the Fluttwerk. Firstly, for every additional enemy, 5s is added to the dance up to 4 enemies. From the 5th enemy onwards, the dance reaches a maximal plateau of 30s. As the enemy details are given to be 7 spice harvesters from the Southwest, the dance will be 30 seconds. When intruded upon by just spice harvesters, only the right wing is fluttered (and for Harkonnen soldiers, the left one is). Distance is not coded for in the dance, so we may ignore it. Lastly for the direction, since it is an approach from the southwest, the abdomen points right.

Q7.

Answer: **FTTT**

Explanation:

A. There was no bottleneck event that led to significant changes in the allele composition/genotypic ratios of the population.
B. This is true as two populations have evolutionarily diverged enough from an ancestral population to form two different clades/species.
C. The chain of Pillars prevented the North and South populations from reproducing with each other, hence preventing gene flow and resulting in speciation.
D. The young Flutter does not instinctually perform the Fluttwerk dance upon the detecting the stimulus.

P23: It’s time for a face lift I

(150 points)

Theropoda is a dinosaur clade characterised by hollow bones and three toes and claws on each limb. They were terrestrial predators of the Late Triassic and became the dominant predators of the land during the Jurassic and Cretaceous. While all dinosaurs are currently extinct, we are still able to investigate their anatomy by making use of fossils.

Muscles work together with bones to produce movement. The function of muscles can be deduced based on their origin and extension. The origin of a muscle refers to the point where the muscle is fixed, while the insertion moves with each contraction. The origin of a muscle is usually attached to the more stable bone, while the insertion is attached to the more mobile bone. Thus, during contraction, the more mobile bone can be brought in closer proximity to the more stable bone.

Sean was on a trip to the Lee Kong Chian National History Museum (LKCNHM) when he spotted several fossils. He is first introduced to Specimen 1. He is shown a video on how the contraction of the jaw muscles of Specimen 1 affects the movement of its jaw. Figure 1 shows the jaw muscles labelled.

Note: For this question participants were provided with a short video clip extracted from the 1min 2s mark to 1min 5s mark of the following video: https://www.youtube.com/watch?v=tdezagMXE2w. The video clip was used with permission.

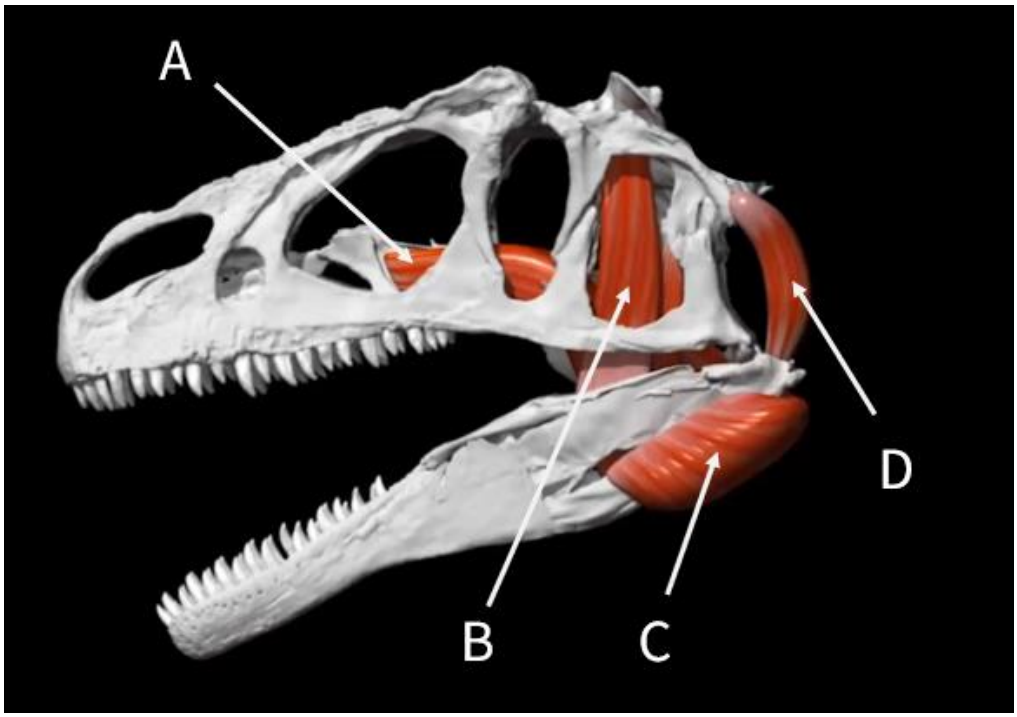


Figure 1: Jaw muscles

Q1. By referring to Figure 1 and the video, help Sean to match the function of the muscle on the jaw to each muscle. Enter *Open* if the muscle opens the jaw and *Close* if the muscle closes the jaw. **(40 points)**

(Enter either "Open" or "Close" to each row.)

Muscle	**Function ("*Open*" or "*Close*")**
A	
B	
C	
D	

The lever action of a musculoskeletal system can be quantified by considering the moment of a force, or torque (τ). The torque can be calculated by the product of the force applied and the perpedicular distance between the point of the applied force and the pivot (Figure 2).

$$\tau = Fd$$

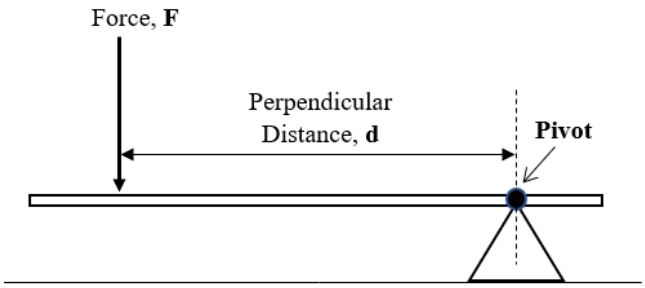


Figure 2: Moment of a force

According to the principles of moments, for a body to remain in rotational equilibrium, the sum of clockwise moments must equal the sum of counterclockwise moments.

Figure 3 shows the lever principle being applied to the human arm. The lever principle governing the mechanics of the human arm also applies to that of the arm of theropods.

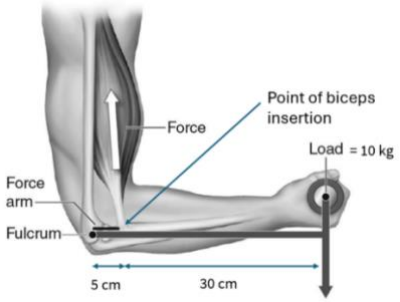


Figure 3: Mechanics of human arm

Q2. Calculate the value of the force exerted by the biceps muscle (in Newtons) in Figure 3 for the system to be in rotational equilibrium. Take gravitational acceleration to be 10 m s^{-2}. **(20 points)**
(Enter your answer correct to nearest whole number. Do not include any units.)

Q3. If a theropod's bicep insertion was 7 cm from the elbow joint and the centre of the hand was 42 cm away from the point of insertion of the biceps, how fast would the object move (in cm s^{-1}) when the biceps shorten 3 cm s^{-1}? **(20 points)**
(Enter your answer correct to nearest whole number. Do not include any units.)

Figure 4 shows several other fossil specimens that Sean saw at the LKCNHM exhibits.

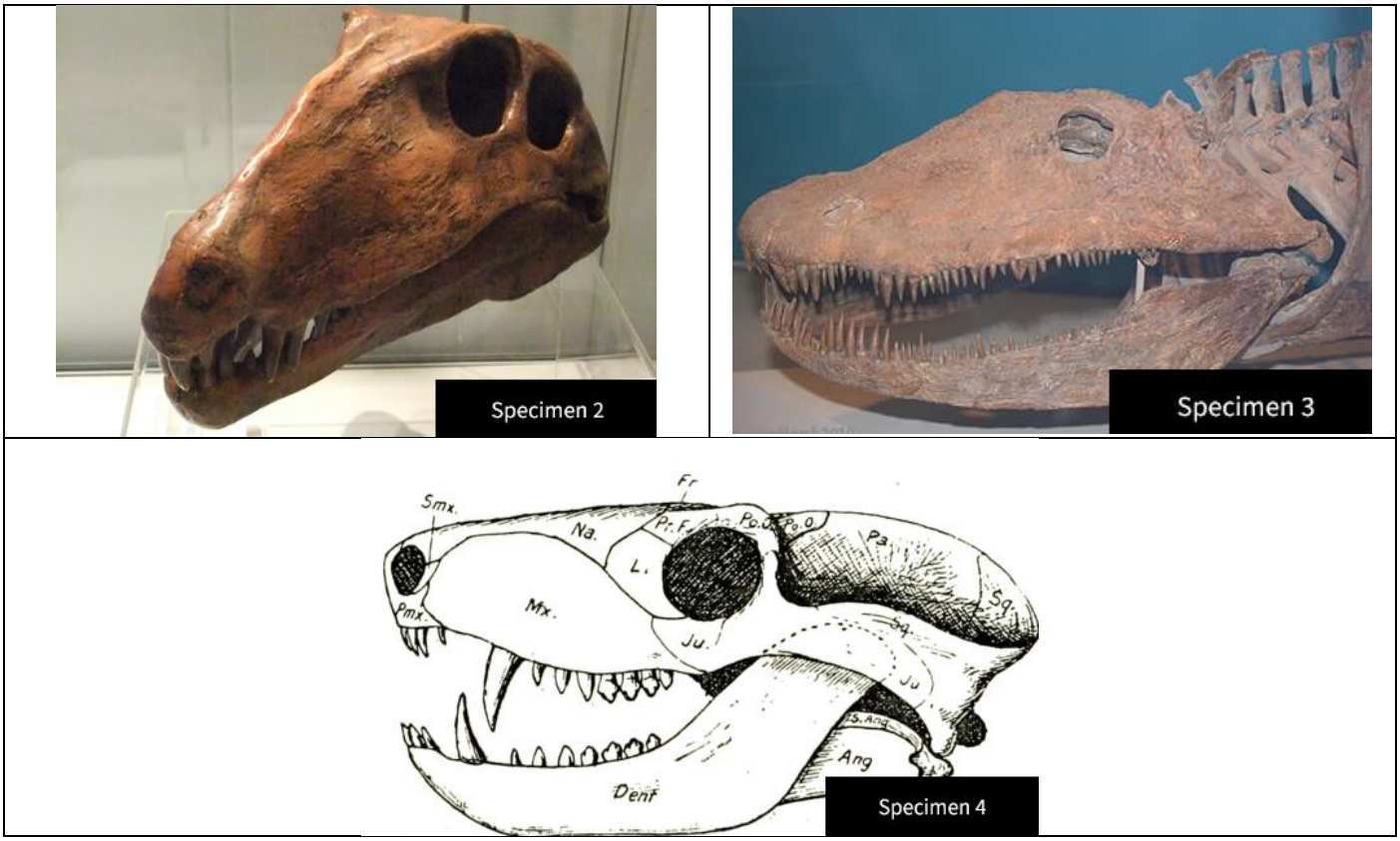


Figure 4: Specimens 2-4

Amniota is a clade of tetrapod vertebrates which includes dinosaurs, birds, and mammals. Amniotes show four different skull types: anapsid, diapsid, synapsid, as well as euryapsid. The skull types are distinguished by the presence and relative location of the temporal fenestrae.

Q4. Based on Figure 4 and the above information, indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. Specimen 1 has three temporal fenestrae.
B. Specimen 2 is a diapsid.
C. Specimen 3 is more likely to be carnivorous than herbivorous.
D. Specimen 3 is heterodontic.

The dental formula is a summary of the arrangement of teeth in an animal. The number of teeth of each type is written as a dental formula for one side of the mouth, or quadrant, with the upper and lower teeth shown on separate rows separated by a slash symbol (/). In each set, the order of teeth is from the outer anterior to inner posterior (Incisor, Canine, Premolar, Molar). The dental formula of adult humans is 2123/2123.

Q5. Indicate the dental formula of the upper and the lower teeth of Specimen 4 and the total number of teeth in the animal. Do not include the slash symbol (/). **(30 points)**
(Enter a number to each row.)

Teeth	**Answer**
Upper teeth	
Lower teeth	
Total Number of Teeth	

Answers and Explanations

Q1.

Answer: **Close, Close, Close, Open**

Explanation:

The functions of A, B and D in opening/closing the jaw can be deduced from the positions of their origins and insertions in the skull. For example, the depressor mandibulae inserts into the posterior end of the mandible, so muscle contraction would result in the mandible swinging downwards, thus opening the jaw. C may be trickier, as the position of its origin is obscured by the mandible, but it can be seen that it closes the skull.

For reference: A: Adductor mandibulae externus, B: Pterygoideus dorsalis, C: Pterygoideus ventralis, D: Depressor mandibulae.

Note: For this question participants were provided with a short video clip extracted from the 1min 2s mark to 1min 5s mark of the following video: https://www.youtube.com/watch?v=tdezagMXE2w. The video clip was used with permission.

Q2.

Answer: **700**

Explanation:

$$W = mg = 10 \times 10 = 100$$

$$5F = 35 \times 100$$

$$F = 700N$$

Remember to multiply mass by gravitational acceleration to obtain the weight of the load, and to take the correct distance between the fulcrum and the load (35 cm, not 30 cm).

Q3.

Answer: **21**

Explanation: Consider what happens when the biceps contract. The forearm now forms the hypotenuse of an imaginary right-angled triangle. By similar triangles, since the hypotenuse of the larger triangle (entire length of forearm; 49 cm) is 7 times the hypotenuse of the smaller triangle (length of forearm between fulcrum and point of biceps insertion; 7 cm), the height of the larger triangle will be 7 times the height of the smaller triangle. Thus, velocity of object movement is 7 times (much faster!) than velocity of muscle shortening.

Q4.

Answer: **FFTF**

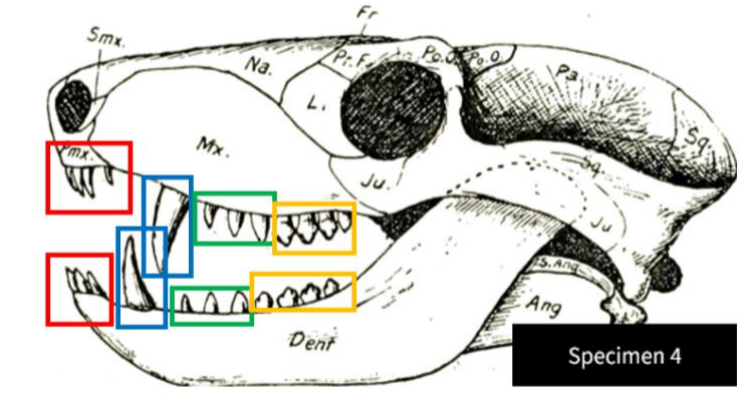
Explanation:

A. Specimen A only has two temporal fenestrae. The anterior hole is actually the orbit.
B. One of the holes is the orbit and the most anterior one is the nasal cavity. Thus, this is actually a synapsid skull with one temporal fenestra.
C. The presence of sharp teeth canine to tear meat implies that it is likely carnivorous.
D. All the teeth appear to be of the same type. Hence, it is likely homodontic.

Q4.

Answer: **4134, 3134, 46**

Explanation: See the diagram below. Red – Incisors, Blue – Canines, Green – Premolars, Orange – Molars. The additional tooth on the upper palate is likely the incisor as it is closer to it and is of similar size.



Credits

The SBL team would like to thank Professor Lawrence M. Witmer from Ohio University for giving us permission to adapt the video at https://www.youtube.com/watch?v=tdezagMXE2w for use in the contest.

Figure 1: WitmerLab (2013, May 21). *Engineering a dinosaur predator - allosaurus feeding mechanics*. YouTube. https://www.youtube.com/watch?v=tdezagMXE2w.

Figure 2: Wallace, L. (2020, December 27). *Moment of a Force*. Physics I Can - Empowerment Through Practice. https://physicsican.blogspot.com/2019/07/moment-of-force.html

Figure 3: *Understand how levers work with your workout*. Human Kinetics Canada. (n.d.). https://canada.humankinetics.com/blogs/excerpt/understand-how-levers-work-with-your-workout

Figure 4:

Specimen 2, Dimetrodon skull: Rept0n1x. (2013, May 21). *Dimetrodon skull, Wrexham Museum, Wales.* Wikipedia. https://upload.wikimedia.org/wikipedia/commons/f/fe/Dimetrodon_skull%2C_Wrexham_Museum.JPG

Specimen 3, Eryops skull: *Eryops sp.* Specimens. (n.d.). https://www.geol.umd.edu/~jmerck/nature/specimens/htmls/eryops53155.html

P24: The Dancing Queen

(140 points)

In unfavourable environmental conditions, bacteria such as *B. subtilis* upregulate the expression of survival genes (e.g. those for formation of endospores). These genes are under negative control by the repressor protein AbrB, which prevents their unnecessary expression. One protein that contributes to the upregulation of survival genes is AbbA, which binds to and blocks AbrB from binding to DNA.

DNA stands for deoxyribonucleic acid, which is made of a phosphate backbone, deoxyribose sugar, and nitrogenous base. The structure of DNA is seen below.

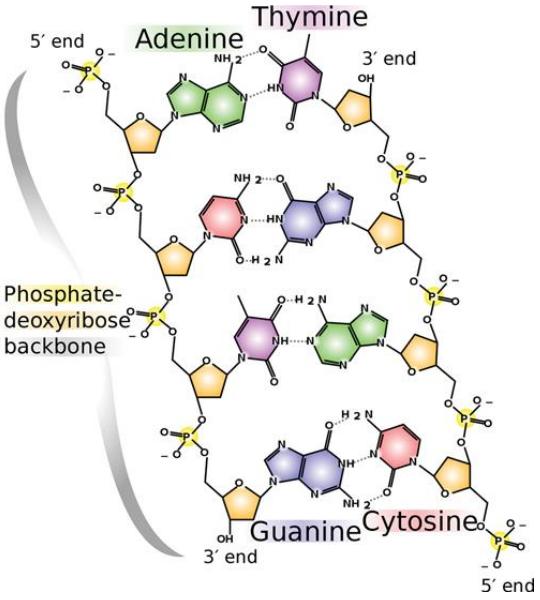


Figure 1: DNA structure

Q1: AbbA forms a dimer and mimics the structure of DNA. Considering the structure of the DNA backbone in Figure 1, which of the following amino acids are likely found in the AbrB-binding region of AbbA? **(20 points)**
(Select all correct options.)

A. Alanine (A)
B. Arginine (R)
C. Aspartic Acid (D)
D. Cysteine (C)
E. Glutamic Acid (E)
F. Isoleucine (I)
G. Lysine (K)
H. Tryptophan (W)

The dimerised nature of AbbA was confirmed via Size-Exclusion Chromatography (SEC) and Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF) Mass Spectrometry. SEC involves passing the solution through a column containing gel beads with small pores. Small molecules pass through the pores and are slowed down while larger molecules bypass the beads and travel straight through the column. In MALDI-TOF, molecules are ionised and travel down a path of fixed length. The ionised molecules are separated by their mass-to-charge (m/z) ratio, with higher m/z ratio travelling slower within the electric field than lighter molecules with lower m/z ratio. Most molecules pick up a singular positive charge from the ionisation process.

Q2. Indicate whether the following statements regarding these two forms of mass spectrometry are true or false. **(40 points)**
(Mark each statement as true or false.)

A. In SEC, an AbbA dimer would pass through the column faster and be eluted earlier than an AbbA monomer.
B. Bio-gel P-60 is appropriate for isolating AbbA and IgG antibodies from a mixture of proteins via SEC.
C. In TOF mass spectrometry, an AbbA dimer would pass through the spectrometer faster and be detected earlier than an AbbA monomer (assuming equal charge).
D. A strong base is required for MALDI-TOF to produce ions containing the sample for analysis.

In SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis), proteins are denatured by the SDS buffer and separated by molecular weight through electrophoresis, so proteins with equal molecular weight (and likely identity) would be found in the same horizontal band of the gel. This technique has been used to investigate AbrB.

Literature shows that four arginine residues (R8, R15, R23, R24) are strongly conserved in AbrB and are critical for its ability to bind DNA. To investigate whether the same residues are necessary for AbrB to bind to AbbA, researchers purified wild-type His_6-AbrB, His_6-$AbrB^{R8A}$, His_6-$AbrB^{R15A}$, His_6-$AbrB^{R23A}$, and His_6-$AbrB^{R24A}$ from bacterial cell lysate. (Note: His_6-$AbrB^{R8A}$ indicates that AbrB has a 6x histidine tag and that R8 is mutant and nonfunctional in this protein.) Chromatography was conducted and the load (L), flow-through (FT), wash (W) and eluate (E) were run through SDS-PAGE and stained with Coomassie Blue.

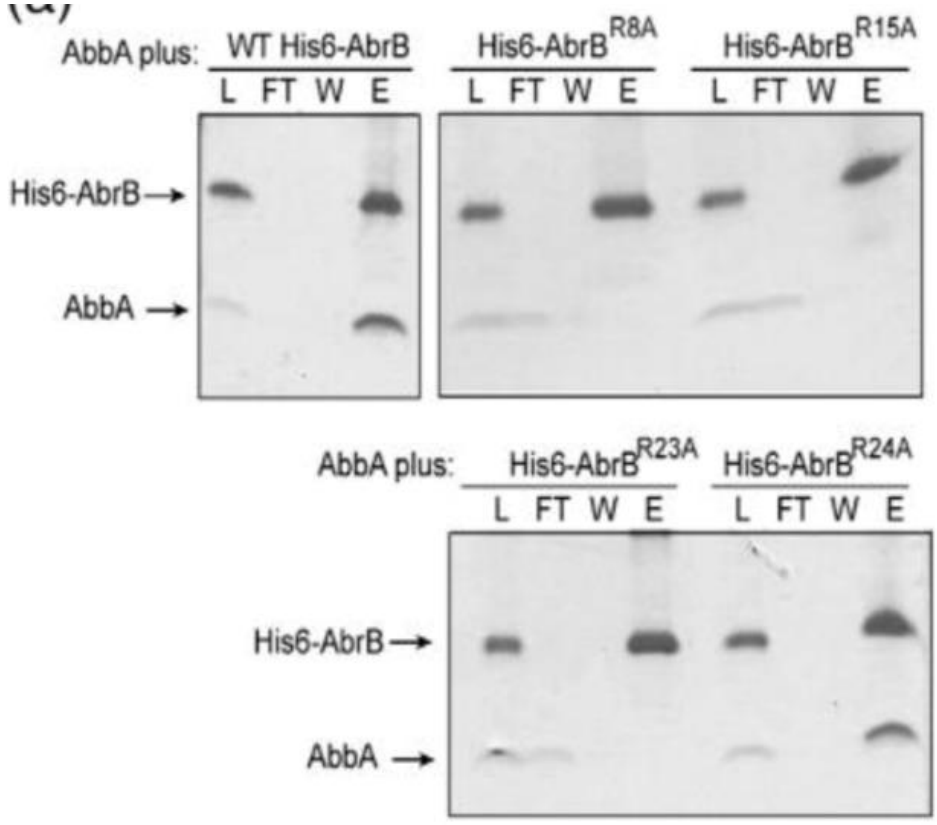


Figure 2: SDS-PAGE gel from chromatography. Coomassie Blue stains proteins.

Q3. What type of chromatography was likely used to purify the His6-AbrB:AbbA complexes? **(20 points)**

(Select the correct option.)

A. Size Exclusion Chromatography
B. Cation Exchange Chromatography
C. Anion Exchange Chromatography
D. Affinity Chromatography
E. High-pressure Liquid Chromatography
F. Thin Layer Chromatography
G. Cannot be deduced

Q4. Which of the arginine residues are necessary for AbbA to bind to AbrB? **(20 points)**

(Select all correct options.)

A. R8
B. R15
C. R23
D. R24

Q5. Indicate whether the following statements regarding the experiment above are true or false. **(40 points)**

(Mark each statement as true or false.)

A. There are traces of AbbA detected in the load solution because some AbbA is still able to bind to AbrB with mutant arginine residues.
B. An imidazole buffer should be used for the wash step because it competes with the His-tagged proteins and prevents unwanted proteins from binding to the column.
C. AbbA is a competitive inhibitor of AbrB.
D. 2D electrophoresis could have been used to produce Figure 2.

Answers and Explanations

Q1.

Answer: **C, E**

Explanation:

The DNA backbone includes many negatively-charged phosphate groups which would attract positively-charged amino acids found at the DNA-binding site of AbrB. Since AbbA mimics DNA, it would likely have the negatively-charged **aspartic acid** and **glutamic acid** at the AbrB-binding site to mimic the negative DNA backbone.

Q2.

Answer: **TFFF**

Explanation:

A. In SEC, large molecules will be eluted from the column before small molecules. Since the AbbA dimer is larger than the monomer, it would pass through the column faster and be eluted earlier.
B. Bio-gel P-60 (produced by Bio-Rad) is effective for proteins of molecular weight between 3kDa and 60kDA. An Abba monomer has molecular weight around 8kDa so both the monomers and dimers would work with P-60, but IgG antibodies have molecular weight of 150kDa and are too large for the gel.
C. Since the AbbA dimer would have double the mass of the monomer, the dimer would have an m/z value twice as large as the monomer, travel slower and be detected later.
D. In general, acids are preferred as the matrix for MALDI-TOF because they can act as a ready source of protons for the ionisation of the sample molecules. Common matrix choices are α-cyano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid and 3,5-dimethoxy-4-hydroxycinnamic acid.

Q3.

Answer: **D**

Explanation: The 6x histidine tag is a common protein sequence that is attached to the N- or C- terminus of the target protein (via adding the histidine-encoding codons to the end of the protein's DNA sequence and expressing it, usually via bacteria). These histidine tags will readily bind to nickel atoms, allowing molecular biologists to extract proteins with histidine tags using **affinity chromatography**, a kind of column chromatography that uses nickel-containing beads. His-tagged proteins bind to the nickel on the beads while other compounds simply wash out of the column. Subsequently, an elution buffer that disrupts the histidine-nickel binding releases the his-tagged proteins from the beads and allows them to be collected in the eluate.

Q4.

Answer: **A, B, C**

Explanation:

The idea behind this experiment is that AbbA-AbrB complexes would bind to the affinity chromatography column because of the His_6-tags on AbrB, while mutants of AbrB that no longer bind to AbbA would cause AbbA to be washed out without binding to the column. Thus, Abba would only be found in the eluate with AbrB mutants that bind to it.

The upper left of the diagram shows that AbbA binds to wild-type AbrB (positive control). For the other four mutants, AbbA only binds to $AbrB^{R24A}$ and is missing from the other three gels, indicating that the mutated amino acids are important for binding to AbbA while R24 is not.

Q5.

Answer: **FTTF**

Explanation:

A. AbbA is detected in the load solution because it must be added to check if it binds to AbrB mutants. The reason the AbbA band is darker in the eluate column is that the eluate contains a high concentration of AbbA-AbrB complexes that were extracted from the chromatography column by the elution buffer, leading to both bands being darker.
B. Imidazole has the same five-carbon ring as histidine that binds well to nickel. Using an imidazole buffer for the wash step would allow the imidazole to outcompete proteins that bind weakly to the nickel aka proteins without the His_6-tag, leaving only the His_6-tagged AbrB (and the bound AbbA) bound to the column.
C. Since the same residues that bind DNA are used to bind AbbA, we know that AbbA competes for the DNA-binding site of AbrB and thus acts as a competitive inhibitor.
D. 2D electrophoresis separates the proteins by their isoelectric point (pI), the pH at which the protein has neutral charge, before separating by molecular weight. The gel comes out with proteins identifiable as distinct spots instead of the bands seen in Figure 2; additionally, it is unlikely that AbbA and AbrB would have near-identical pI values.

Credits

Figure 1: Roberts, M. A. (2019). Recombinant DNA technology and DNA sequencing. *Essays in Biochemistry*, *63*(4), 457–468. https://doi.org/10.1042/ebc20180039

Figure 2: Tucker, A. T., Bobay, B. G., Banse, A. V., Olson, A. L., Soderblom, E. J., Moseley, M. A., Thompson, R. J., Varney, K. M., Losick, R., & Cavanagh, J. (2014). A DNA mimic: the structure and mechanism of action for the anti-repressor protein AbbA. *Journal of molecular biology*, *426*(9), 1911–1924. https://doi.org/10.1016/j.jmb.2014.02.010

P25: Potato Disease

(180 points)

The Potato Disease (PDT) is a rare but completely-penetrant disease found in individuals in the country of BioMania. PDT is caused by a single transversion mutation in the 5200bp-long gene *potate*. This mutation causes the face to turn yellow and the brain to swell up. This causes encephalitis and meningitis, which quickly results in death within two years that symptoms manifest.

PDT is usually diagnosed by genetic testing of a microsatellite region situated sufficiently close to the gene that it is effectively completely linked. Figure 1 shows the normal and mutant alleles and their respectively linked microsatellite.

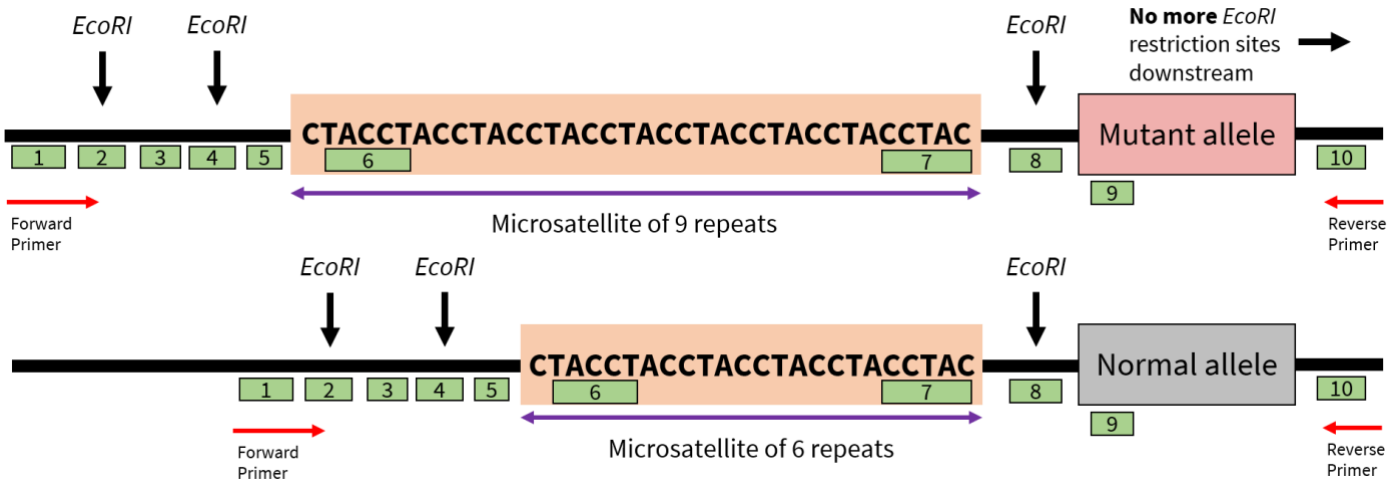


Figure 1: Map of *potate* gene and its linked microsatellite. *EcoRI* restriction sites are indicated. No *EcoRI* restriction sites can be found in all the 2 million base pairs downstream of the *potate* gene. Green boxes indicate possible radioactive probes.

These microsatellite regions are situated between two *EcoRI* restriction sites as seen in Figure 1. To perform the protocol, Jason first performed PCR using primers as indicated in red in Figure 1. He then cleaved the DNA fragment using *EcoRI*, removed the *EcoRI* enzymes, and then incubated the DNA fragments with radioactive probes to produce the result in Figure 2. He did so for Generations I and II but did not do so for Individual III-1 as the child was too young. However, as Joseph was a potato, he forgot to label the lanes and had no clue which lane corresponded to which individual (*bruh*).

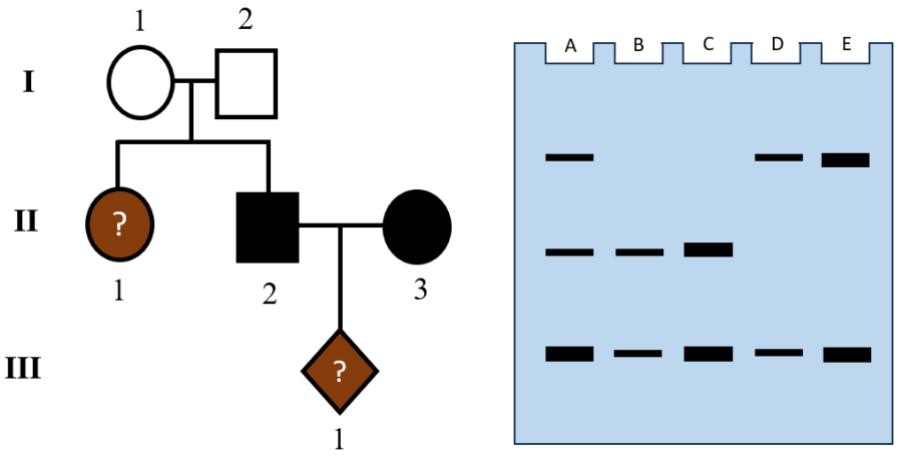


Figure 2: (left) Pedigree of a family with PDT. White indicates that the individual does not have PDT while black indicates that the individual is affected. However, it is unknown whether Individuals II-1 and III-1 are affected. (right) Gel electrophoresis of the samples of Generations I and II after incubation with radioactive probes

Q1. You used only one type of probe to conduct the experiment. Which probes, when used individually, could have been used to produce the result in Figure 2? **(30 points)**
(Select all correct options.)

A. Probe 1
B. Probe 2
C. Probe 3
D. Probe 4
E. Probe 5
F. Probe 6
G. Probe 7
H. Probe 8
I. Probe 9
J. Probe 10

Q2. What is the nucleotide sequence of the microsatellite repeat? **(10 points)**
(Enter a string of letters. Do not include 5' or 3'.)

Q3. What is the mode of inheritance of PDT? **(20 points)**

(Select the correct option.)

A. Autosomal recessive
B. Autosomal dominant
C. X-linked recessive
D. X-linked dominant
E. Y-linked
F. Paternal imprinting
G. Maternal imprinting
H. Cytoplasmic inheritance

Q4. Indicate whether the following statements are true or false. **(50 points)**

(Mark each statement as true or false.)

A. The mutation could have been a change from cytosine to thymine.
B. The mutant allele is a lethal allele.
C. Individual II-1 is unaffected but is a carrier.
D. Lane B corresponds to Individual II-3.
E. PDT is unlikely to develop symptoms below 10 years of age.

Q5. What is the probability that Individual III-1 will develop PDT? **(10 points)**

(Enter your answer as a decimal correct to 3 s.f.)

Q6. Individuals I-1 and I-2 decide to have ten more children in addition to the two they already have. What is the probability that exactly four of the ten children subsequently develop PDT? **(30 points)**

(Enter your answer as a decimal correct to 3 s.f.)

A similar disease, *patata*, is inherited in an autosomal dominant manner. A variable number tandem repeat (VNTR) is found near to the gene locus that causes *patata*.

Figure 3 shows a pedigree with affected individuals shaded black and their respective VNTRs indicated. There are seven different VNTRs (V1 to V7).

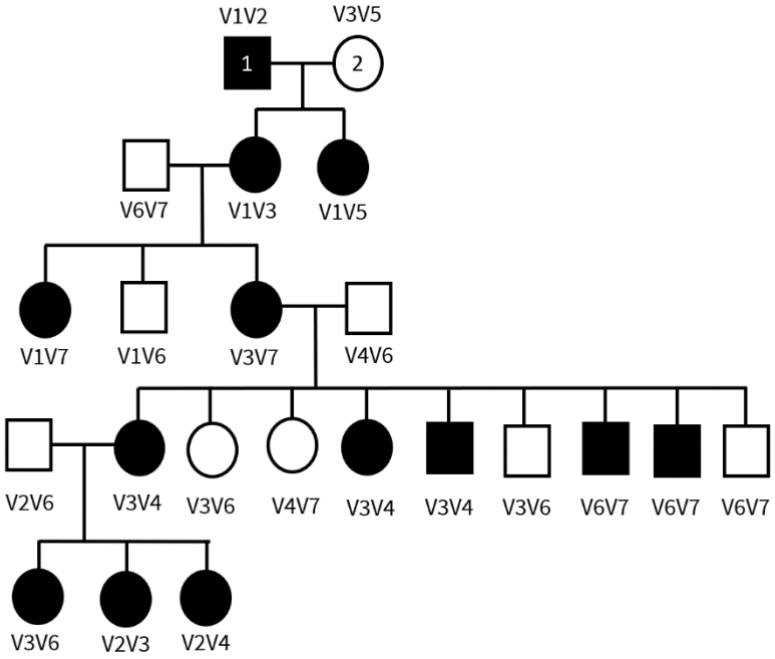


Figure 3: Pedigree of *patata* in a family. Affected individuals are shaded black. The respective VNTRs are indicated. Individuals 1 and 2 are known to be homozygous.

Q7. Based on Figure 3, calculate the map distance between the VNTR locus and the *patata* gene locus in map units (m.u.). **(30 points)**
(Enter your answer correct to 3 s.f. Do not enter any units.)

Answers and Explanations

Q1.

Answer: **D**

Explanation: *EcoRI* is added to the mixture which cleaves the DNA at its restriction sites. We are investigating the presence of the microsatellite as an indicator of the presence of either allele, so we should be choosing a probe that anneals to the microsatellite fragment produced after *EcoRI* digestion (as represented by the uppermost and intermediate bands in Figure 2). Hence, the probes in consideration are Probes 4 to 8. However, we also notice a bottommost band in Figure 2. This implies that the probe must bind to two separate fragments so as to produce the band, hence only Probes 4 and 8 are possible. However, we note that the *potate* gene is 5200bp long, so if Probe 8 is used, the band that is present in all individuals should be closer to the wells as it is very heavy as compared to the microsatellite fragment. Thus, only Probe 4 could have been used.

Q2.

Answer: **CTAC**

Explanation: It is clear from Figure 1 that the microsatellite repeat is CTAC. The repeat is repeated nine times on the mutant homologue and four times on the normal homologue.

Q3.

Answer: **C**

Explanation: We see that certain individuals in Figure 2 have a lighter (less intense) band and others have a darker (more intense) band. This implies that some individuals have more copies of the fragment. As PCR was conducted the same number of times for each individual, this could only be explained by an innate difference in the number of genes. Hence, the disease is a sex-linked disease, which explains why males have a lighter (less intense) band as they have only one X-chromosome. As heterozygosity is possible, as indicated by the presence of both bands in Individual A, this must be an X-linked disorder. We also observe a skipping of generation from Generation I to Generation II, so this must be a recessive disorder. Hence, the mode of inheritance is X-linked recessive. Imprinting does not satisfactorily explain the difference in band intensities, while cytoplasmic inheritance would mean that II-2 cannot be affected as I-1 is unaffected. Hence, these are not possible answers.

Deducing which Individual belong to which Gel Lane

We can first solve the pedigree. Since it is an X-linked recessive disorder, Individual I-1 must be heterozygous so as to pass on one mutant allele to II-2. Heterozygosity is indicated by the presence of both bands, so **Lane A belongs to I-1**. Next, I-2 is unaffected and is a male, so he must have the normal allele. Since the microsatellite of the normal allele is shorter than that of the mutant allele, the band should appear further from the wells as it is less impeded by the agarose gel. As he is a male, he only has one X chromosome, hence his band will be lighter (less intense). Hence, **Lane D belongs to I-2**. II-2 is an affected male so consequently **Lane B belongs to II-2**. Individual II-3 is affected, so she must be homozygous for the recessive allele. Hence, she must have a darker (more intense) band which is closer to the wells, and **Lane E belongs to II-3**. By the process of elimination, **Lane C belongs to II-1**, and she is homozygous dominant due to the presence of only one band further from the wells, so she has two copies of the normal allele. Hence, she is unaffected.

Q4.

Answer: **FTFFT**

Explanation:

A. Since PDT is caused by a single transversion mutation, the base substitution must be from a purine to pyrimidine or vice versa. As cytosine and thymine are both pyrimidines, this substitution cannot be a transversion mutation.
B. Since the mutant allele ultimately causes in the death of the individual, the allele is a lethal allele.
C. We have already deduced above that Individual II-1 is a homozygote and is unaffected.
D. We have already deduced above that Lane B belongs to II-2.
E. PDT causes death within two years that symptoms manifest. We observe that affected individuals II-2 and II-3 are able to live to reproductive age to produce Individual III-1, so symptoms cannot manifest before reproductive age or else the allele cannot be passed down via homozygous recessive individuals as they would not have been able to live to that age.

Q5.

Answer: **1.00**

Explanation: Since both parents II-2 and II-3 are affected, only recessive alleles on the X chromosome can be passed to the child regardless of the sex of the child, hence the child must be affected.

Q6.

Answer: **0.146**

Explanation: Individual I-1 and I-2 are heterozygous and hemizygous for the normal allele respectively. As each birth is an independent event, the genotypes of II-1 and II-2 will not affect the required probability. The probability that one offspring develops PDT is 0.25, as seen in the Punnett square below:

	X^A	Y
X^A	X^AX^A	X^AY
X^a	X^AX^a	X^aY

The probability that exactly four of ten children develop PDT can be calculated as follows:

$$\boldsymbol{P(required) = \frac{10!}{4!\,(10-4)!}\; P(4\;affected)\; P(6\;unaffected)}$$

This can be interpreted as finding the probability that exactly four children is affected and six children is unaffected, which would give four of ten affected children. The $\frac{\mathbf{10!}}{\mathbf{4!(10-4)!}}$ term represents the number of ways to choose four children out of the ten children to be the affected one. This is because only Individuals A-D being affected is not the same as only Individuals B-E or C-F being affected. This coefficient can also be expressed using the choose function ${}^{10}C_4$. Since we could also be choosing six of the ten children to be unaffected, we can also use the equivalent coefficient $\frac{\mathbf{10!}}{\mathbf{6!(10-6)!}}$ Or ${}^{10}C_6$.

Thus, the required probability is:

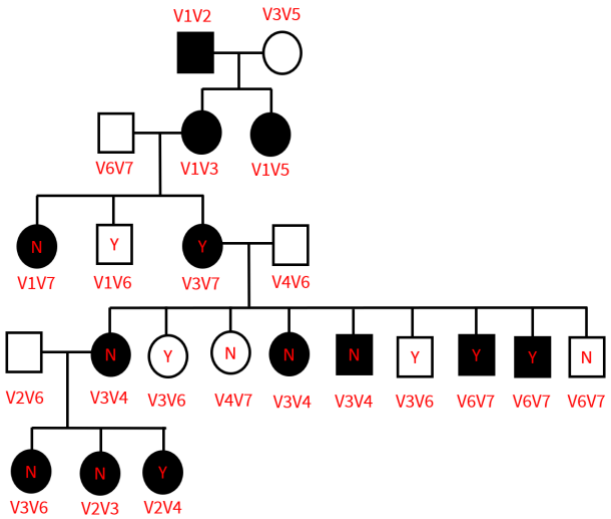
$$\boldsymbol{P(required) = \frac{10!}{4!\,(10-4)!}\;(0.25^4)\;(0.75^6) = 0.146}$$

Q7.

Answer: **46.7**

Explanation: To calculate the map distance, we need to find out the degree of recombination at the locus between the VNTR and the *patata* allele. This can be found through the pedigree in Figure 3. Individuals who receive the VNTR linked with the *patata* allele should be affected with *patata*. However, if they are unaffected, recombination must have occurred which unlinked the VNTR and the *patata* allele, so the individual now has the "diseased" VNTR but it is linked to the normal allele. Since this disease is inherited in an autosomal dominant manner, individuals must be affected as long as they have one mutant allele.

Using this logic, we can find which individuals have had recombination occur. In Generation I, we do not know which VNTR, 1 or 2, is linked to the mutant allele, but we know that both V3 and V5 are not. Looking at Generation II, it is clear that minimally V1 is linked to the diseased allele as both offspring in have V1 and are affected. While it is possible that recombination had occurred in both individuals and V2 was actually linked to the diseased allele, this does not matter as there is insufficient evidence so this cannot be used in the calculations, and even so V1 would be regarded as linked to the mutant allele to be passed on to all the progeny. In Generation III, we would expect individuals who receive V1 to be affected and those who receive V3 to be unaffected. Any deviation represents a recombination event. In the diagram below, "Y" represents that recombination occurred while "N" represents that recombination did not occur. This can be continued down the pedigree.



We count there to be 7 recombinant events, and the number of individuals sampled is 15. Hence the degree of recombination and hence map distance is:

$$\boldsymbol{Map\ distance = \frac{Recombinants}{Total\ number\ of\ individuals} = \frac{7}{15} \times 100 = 46.7\ m.u.}$$

P26: Abs

(130 points)

Abscisic acid (ABA) is known to regulate dormancy of seeds and to regulate other stress responses in plants. Phytochromes are light photoreceptors that are involved in photoperception of the light environment. Faith hypothesises that phytochromes regulate the signalling of ABA in plants. She decides to investigate how phytochrome A (phyA) and phytochrome B (phyB) regulate ABA signalling in *Arabidopsis*.

Faith sowed wild-type (WT), *phyA* mutant (*phyA*$^-$), and *phyB* mutant (*phyB*$^-$) seeds on medium containing various concentrations of ABA and grew them vertically in R light for 5 days. The results are seen in Figure 1A. Then, she sowed the seeds of WT, *phyA* mutant, and two *phyA* complementation lines on medium containing various concentrations of ABA for 1 day in white light, and then transferred to FR light and incubated for additional 6 days. The results are seen in Figure 1B.

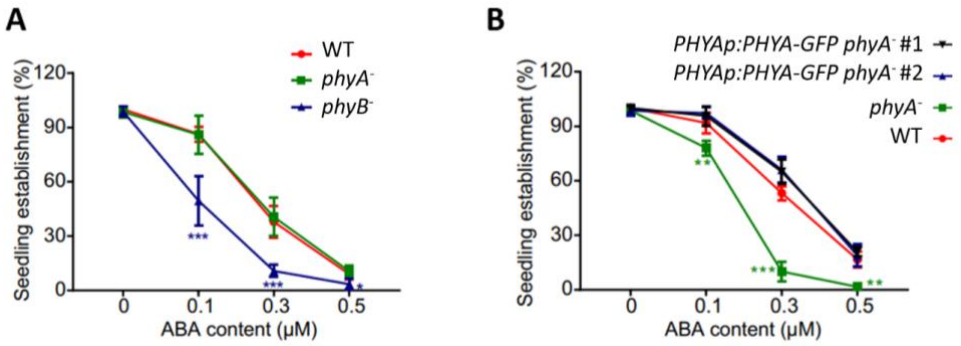


Figure 1: ABA on *phyA*$^-$ and *phyB*$^-$ mutants' germination.
(A) Seedling establishment rates of WT, *phyA*$^-$, and *phyB*$^-$ seedlings grown on medium containing different concentrations of ABA in R light for 5 days.
(B) Seedling establishment rates of WT, *phyA*$^-$, and two *phyA*$^-$ complementation lines grown vertically on the medium containing the indicated concentrations of ABA in white light for 1 day, then transferred to FR light, and incubated for an additional of 6 days. Complementation lines contained *phyA*-GFP under the control of the native PHYA promoter.

Q1. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. *phyA* has negligible effect on the regulation of ABA signalling in R light but *phyB* positively regulates ABA signalling in R light.
B. *phyA* may be epistatic to *phyB*.
C. *phyA* mutant seeds show low germination rates under FR light in the presence of ABA.
D. *phyA* mutant seeds become more sensitive to ABA if *phyA*-GFP is introduced under the control of a native PHYA promoter.

To further investigate the effects of phyA and phyB on ABA signalling, Faith created an ABA system in yeast cells that emulates the ABA-signalling pathway, with a downsteam transcription factor that controls a reporter. She then fused a nuclear localisation signal (NLS) with phyA, phyB or COP1 (phyA-NLS, phyB-NLS, NLS-COP1 respectively) and they were expressed in these yeast cells. PCB was added as the substrate for phytochromes. In the presence of R or FR light, the phyA and phyB proteins can thus convert into their Pr or Pfr forms. The results are shown in Figure 2A-C.

In addition, she also tested the effect of a phyA point mutation phyA #3. The results are shown in Figure 2D. Figure 2E shows the results when one of the components of the emulated ABA-signalling pathway, KT8, is substituted with homologous KE7 and KB9 respectively. Figure 2F shows the results when ABI1 in the pathway is replaced with homologous ABI2.

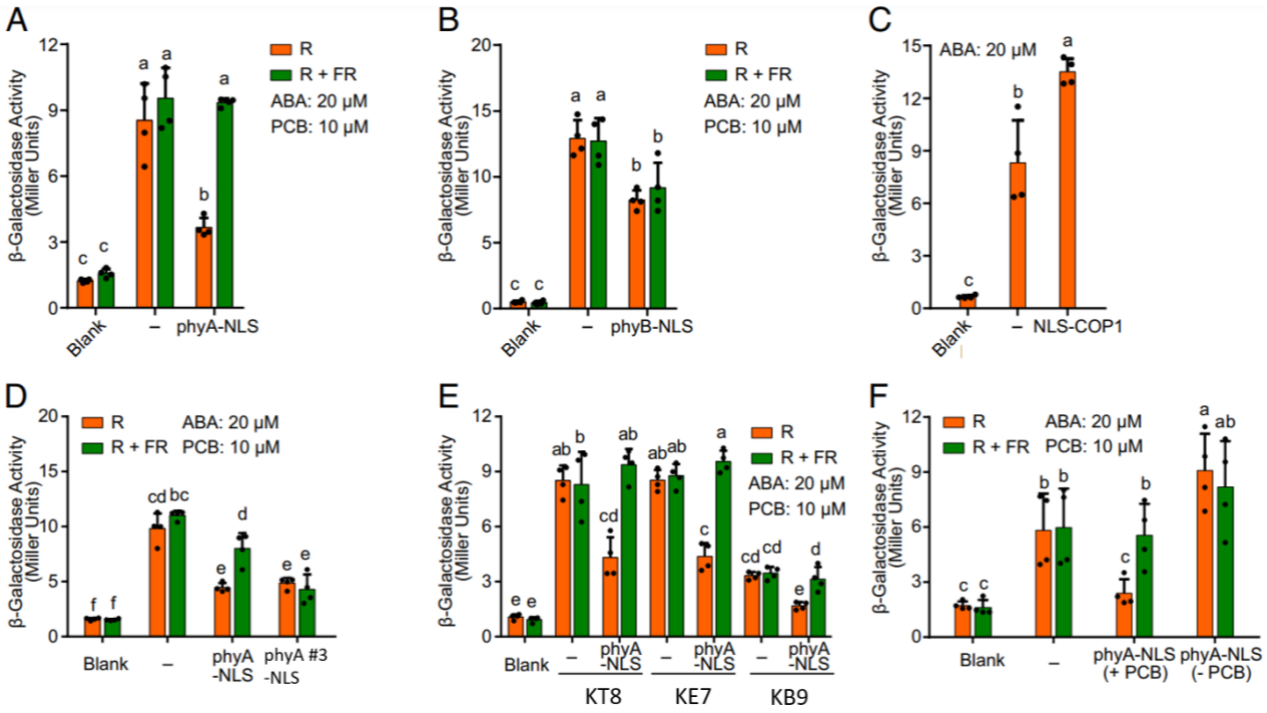


Figure 2: phyA, phyB, and COP1 effects on ABA signalling in yeast cells.

(A-C) phyA (A), phyB (B), and COP1 (C) effect on ABA signalling.

(D) phyA and mutant phyA #3 on ABA signalling

(E) Effects on ABA signalling after substitution of KT8 with KE7 or KB9.

(F) Effects on ABA signalling after substitution of ABI1 with ABI2 and in the presence or absence of PCB.

Q2. Indicate the effect of the following proteins on the regulation of ABA signalling in the transformed yeast cells by matching the following options (1-3) to the correct row. **(50 points)**
(Match the correct number to the correct row.)

1. Positive
2. Negative
3. Negligible

Protein	**Effect (1-3)**
Pr form of phyA	
Pfr form of phyA	
Pr form of phyB	
Pfr form of phyB	
COP1	

Q3. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. phyA #3 likely contains a mutation that inhibits the conversion of Pfr to Pr.
B. phyA affects the ABA signalling pathway when KT8 is substituted with KB9 but not when KT8 is substituted with KE7.
C. phyA still inhibits the ABA signalling pathway even after ABI1 is substituted with ABI2.
D. The predominant effect of phyA on ABA signalling is via the Pfr form.

Answers and Explanations

Q1.

Answer: **FFTF**

Explanation:

A. ABA signalling inhibits seedling establishment and the effects are most pronounced in *phyB*$^{-}$ mutant in R light. Comparing wild-type and *phyB*$^{-}$ mutants, the effect of ABA signalling inhibiting seedling establishment indicates that *phyB* **negatively** regulates ABA signalling in R light.
B. As seen in Figure 1A, *phyA*$^{-}$ mutants did not show any difference in seedling establishment compared to WT, while *phyB*$^{-}$ mutants showed a fall in seedling establishment. This suggests that it is possible that *phyB* is epistatic over *phyA*, which is why there is only a change in phenotype when *phyB* was mutated. However, it is unlikely that *phyA* was epistatic over *phyB* as there was no effect on seedling establishment after *phyA* was mutated.
C. There is a significantly lower seedling establishment rate for *phyA*$^{-}$ mutants compared to wild-type at all ABA concentrations as seen in Figure 1B.
D. There is no significant difference between wild type and *PHYAp:PHYA-GFP phyA*$^{-}$ in response to ABA in Figure 1B.

Q2.

Answer: **3, 2, 2, 2, 1**

Explanation:

A. A higher β-galactosidase activity will indicate increased ABA signalling. Red light converts the phytochrome into the Pfr form and far-right light converts the phytochrome into the Pr form.
B. As seen in figure 2A, under R followed by FR light, Pr form of phyA is active. Since the β-galactosidase activity is identical to the control, Pr form of phyA has a negligible effect on ABA signalling.
C. As seen in figure 2A, under R light, Pfr form of phyA is active. Since the β-galactosidase activity is lower than the control, Pr form of phyA has a negative effect on ABA signalling.
D. As seen in Figure 2B, under R followed by FR light, Pr form of phyB is active. Under R light, Pfr form of phyB is active. Since the β-galactosidase activity is lower than the control, Pr and Pfr form of phyB has a negative effect on ABA signalling.
E. As seen in Figure 2C, since the β-galactosidase activity is higher than the control, Pr and Pfr form of phyB has a positive effect on ABA signalling.

Q3.

Answer: **TFTT**

Explanation:

A. Comparing phyA and phyA #3 in figure 2D, phyA #3 under both red and far-red light behaves as phyA under red light (Pfr form of phyA). This may be explained by the inability of phyA #3 to be converted back to the Pr form from the Pfr form.
B. In figure 2E, Pfr form of phyA negatively affects the ABA signalling pathway compared to control regardless whether KT8 is substituted by KE7 or KB9.
C. Pfr form of phyA still negatively affects the ABA signalling pathway even after substitution of ABI1 with ABI2.
D. Pfr form of phyA is active as it negatively affects the ABA signalling pathway under red light. In contrast, the Pr form of phyA does not have any significant effect.

Credits

Figures 1 and 2: Li, H., Zhou, Y., Qin, X., Peng, J., Han, R., Lv, Y., Li, C., Qi, L., Qu, G.-P., Yang, L., Li, Y., Terzaghi, W., Li, Z., Qin, F., Gong, Z., Deng, X. W., & Li, J. (2023). Reconstitution of phytochrome A-mediated light modulation of the ABA signaling pathways in yeast. *Proceedings of the National Academy of Sciences*, *120*(34). https://doi.org/10.1073/pnas.2302901120

P27: It’s time for a face lift II

(150 points)

Sean continues his trip in the Lee Kong Chian National History Museum (LKCNHM). He sees Figure 1, which shows the evolution of cynodonts and mammaliaforms as well as their dietary adaptations and modifications in skull morphology. He also sees Figure 2, the skull of *Pascualgnathus*.

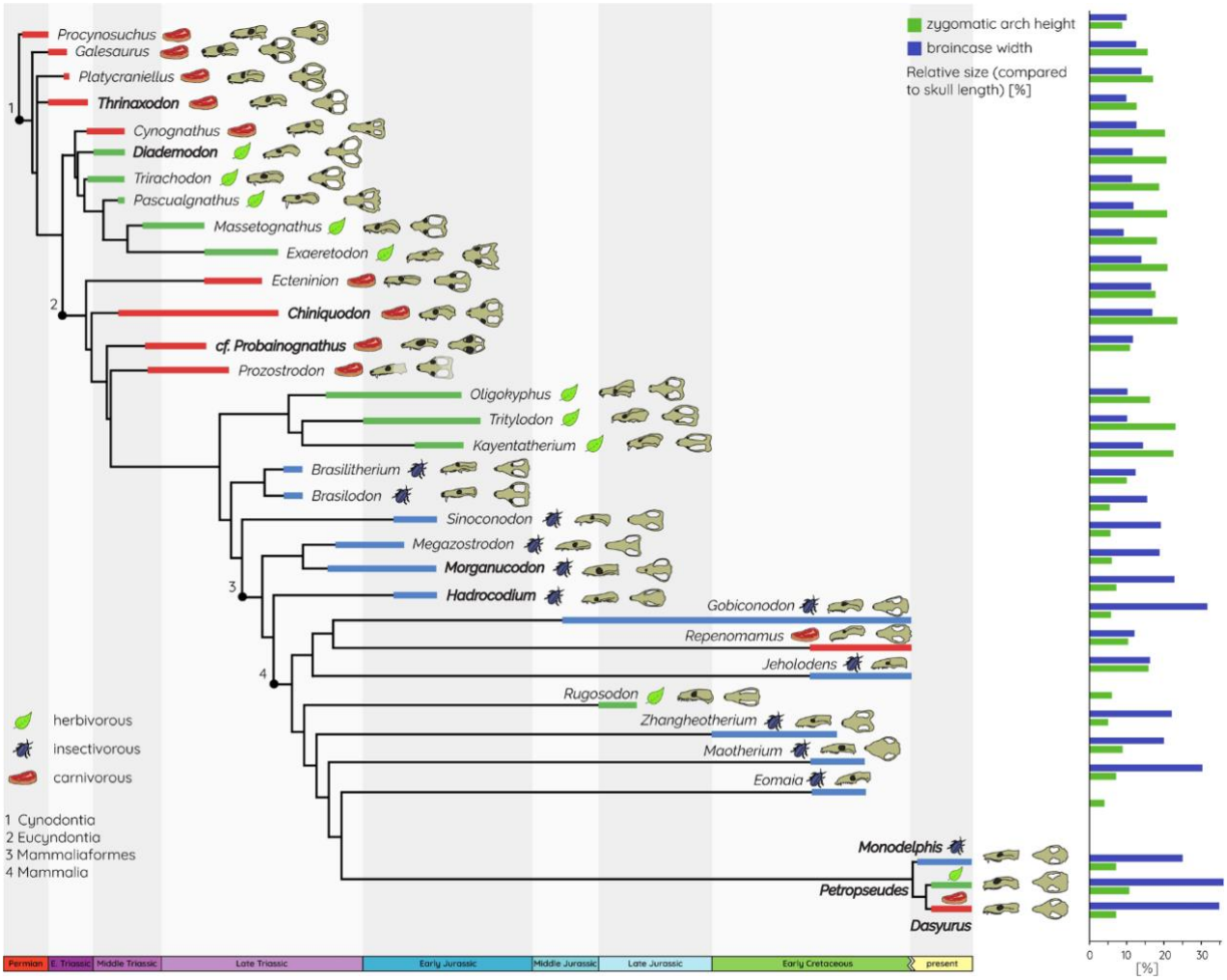


Figure 1: Evolutionary of cynodonts and mammaliaforms.

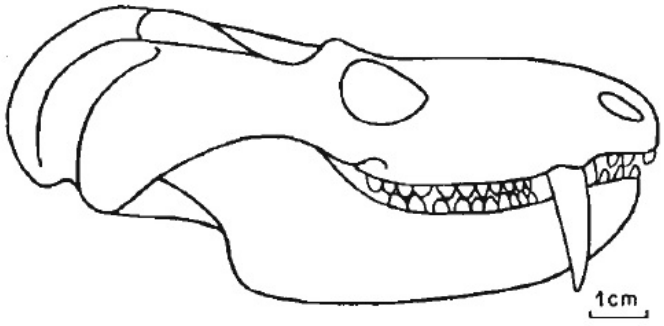


Figure 2: *Pascualgnathus* skull

Q1. By referring to Figure 1, indicate whether the following statements are true or false. **(40 points)**

(Mark each statement as true or false.)

A. The changes in the relative sizes on the right-hand side of Figure 1 could have been driven by a selective advantage in stronger skulls.
B. Most cynodonts have a zygomatic arch length of 5% to 15% of the skull length.
C. *Eomaia* and *Maotherium* are sister taxa.
D. Herbivory is an autapomorphic trait in *Rugosodon*.

Q2. By referring to Figure 1, indicate whether the following statements are true or false. **(40 points)**

(Mark each statement as true or false.)

A. The Mammalia clade likely underwent adaptive radiation.
B. The extinct species in Figure 1 form a monophyletic clade.
C. The dental arrangement of *Pascualgnathus* is unusual considering its diet.
D. The most recent common ancestor of *Rugosodon* and *Brasilitherium* is more recent than the most recent common ancestor of *Zhanghoetherium* and *Brasilodon*.

Occam's razor is a principle used in phylogenetics to find the most parsimonious phylogenetic tree. Occam's razor states that if hypotheses have equal explanatory powers, the one requiring the fewest assumptions should be the preferred hypothesis. Hence, with all other things being equal, the phylogenetic tree which requires the fewest number of evolutionary changes is the best hypothesis and can be used as the most parsimonious tree.

Q3. Using Occam's razor, calculate the most likely number of diet changes that occurred in the phylogenetic tree in Figure 1. **(10 points)**

(Enter a whole number.)

Fossils are also useful in helping to determine the relative ages of different strata. They are called index fossils. Figure 3 shows several outcrops taken from different parts of the world containing different fossils.

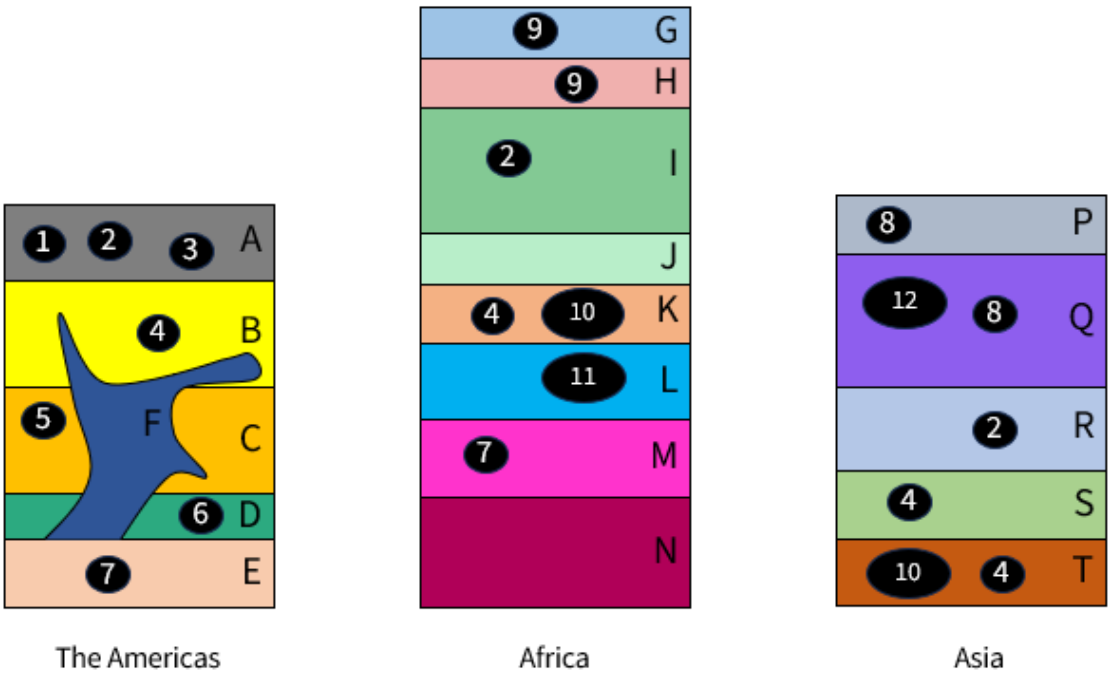


Figure 3: Outcrops. Fossils are in numbers (1-12) and rock layers are in letters (A-T). To avoid confusion no layer is labelled O.

Q4. Indicate which fossil (1-12) should be used as the index fossil. **(10 points)**
(Enter a whole number.)

Q5. Indicate whether the following statements are true or false. **(50 points)**
(Mark each statement as true or false.)

A. Since Asia has a fewer number of strata as compared to Africa, we can deduce that Africa existed before Asia.
B. Fossil 3 is older than Fossil 9.
C. Layer F is older than Layer B.
D. Layer J is younger than Layer S.
E. Lava likely flowed onto the surface in Layer J but not Layer R.

Answers and Explanations

Q1.

Answer: **TFFF**

Explanation:

A. A selective advantage in stronger skulls would mean that braincase width rises and zygomatic arch height decreases. This is seen in Figure 1 as over time, such skulls become more prevalent.
B. Cynodonts have a zygomatic arch length of 20% to 30% of the skull length.
C. (((*Dasyrus*, *Petropseudes*), *Monodelphis*), *Eomaia*) and *Maotherium* are sister taxa.
E. Herbivory is not an autamorphic trait in *Rugosodon* as it is not unique to *Rugosodon* and is present in other species such as *Oligokyphus* and *Trirachodon*.

Q2.

Answer: **TFTF**

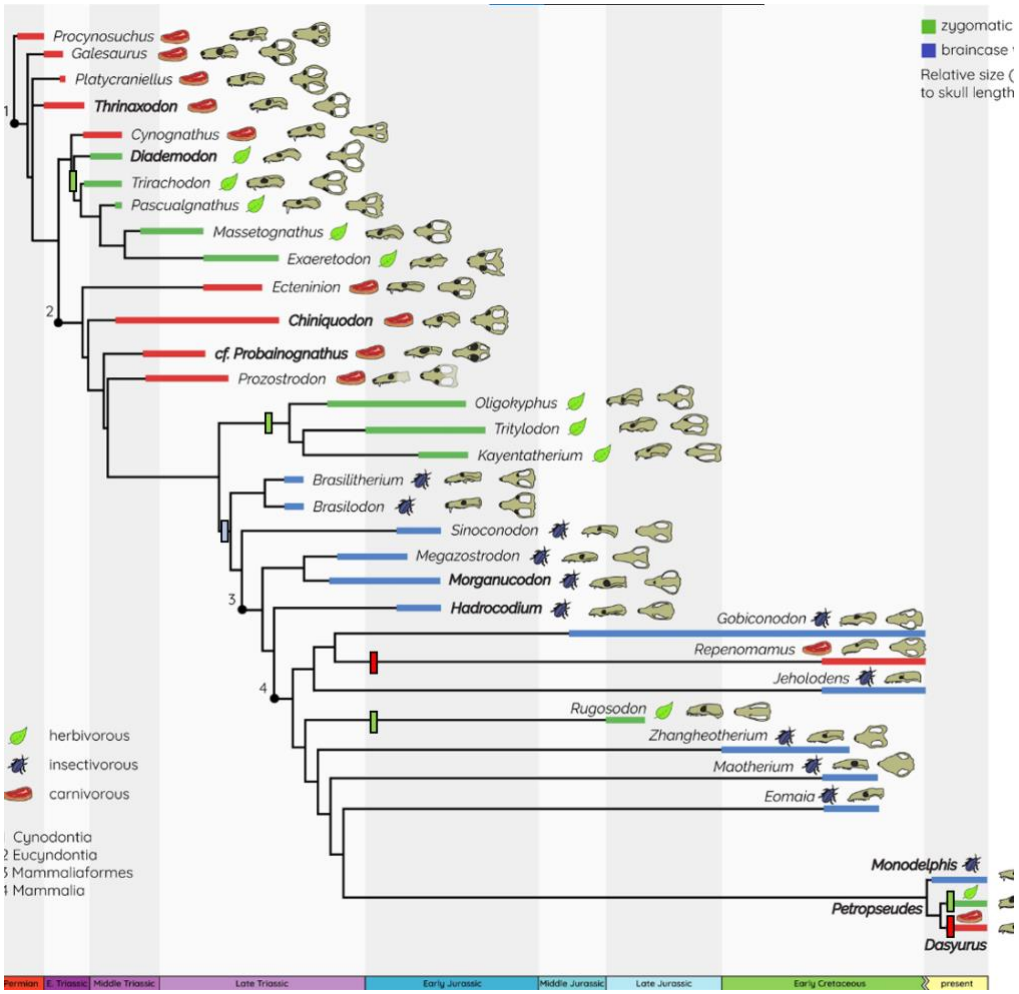
Explanation:

A. By occupying different niches the *Mammalia* clade rapidly evoled and developed different adaptations to their unique environments.
B. The extinct species form a paraphyletic clade as not all the descendants are included i.e. the extant species.
C. *Pascualgnathus* is a herbivore yet it has a sharp canine. This is unusual as canines are used to tear apart meat. We can also see that the canine is selected against and becomes smaller in other herbivorous clades.
D. Both most recent common ancestors are the same at the same node.

Q3.

Answer: **7**

Explanation: The seven changes can be seen below as indicated by the coloured rectangles.



Q4.

Answer: **2**

Explanation: It is present in all three outcrops and is only present in one layer in each outcrop (A, I, R). Hence it is widespread but short-living and can be used to date the layers.

Q5.

Answer: **FTFFT**

Explanation:

A. The number of strata reflects local geological processes over millions of years but does not directly correlate with the age of the continent and cannot be used to compare the ages across outcrops.
E. Using 2 as an index fossil, A and I both have the same geological age, and hence layer H and consequently Fossil 9 is younger than Fossil 3 which is in Layer A.
F. Layer F represents an intrusion of lava into the layers and is hence younger than Layer B.
G. This cannot be deduced as they are both present in the layer below the layer containing the index fossil.
H. Lava likely extruded which caused all the fossils to be lost. The lack of fossils in Layer J hence suggests that lava was extruded, while the presence of lava in Layer R suggests that no lava was extruded.

Credits

Figure 1: Lautenschlager, S., Fagan, M. J., Luo, Z.-X., Bird, C. M., Gill, P., & Rayfield, E. J. (2023). Functional reorganisation of the cranial skeleton during the cynodont–mammaliaform transition. *Communications Biology*, *6*(1). https://doi.org/10.1038/s42003-023-04742-0

Figure 2: Battail, B. (1983). La phylogenie des Cynodontes gomphodontes. *Acta Palaeontologica Polonica*, *28*(1–2), 19–30.

P28: X marks the spot

(130 points)

After much X-raying and crossbreeding, Dr Pahari has created a pure-breeding *Drosophila melanogaster* with genotype $bbprprvgvgss$, where the gene loci of b, pr, vg and s are all found on the same chromosome. Excited, he crossed it with a wild-type strain with $b^+b^+pr^+pr^+vg^+vg^+s^+s^+$ genotype and performed a test cross of the F_1 generation. The + sign indicates the wild-type allele, while the lack of the + sign indicates the mutant recessive allele.

Legend	
b^+: black body	b: grey body
pr^+: red eyes	pr: purple eyes
vg^+: normal wings	vg: vestigial wings
s^+: long bristles	s: short bristles

The results are shown in the table below.

Phenotype	**Number**
Black body, red eyes, normal wings, long bristles	411
Black body, red eyes, normal wings, short bristles	409
Black body, red eyes, vestigial wings, long bristles	61
Black body, red eyes, vestigial wings, short bristles	58
Black body, purple eyes, normal wings, long bristles	2
Black body, purple eyes, normal wings, short bristles	2
Black body, purple eyes, vestigial wings, long bristles	30
Black body, purple eyes, vestigial wings, short bristles	28
Grey body, red eyes, normal wings, long bristles	28
Grey body, red eyes, normal wings, short bristles	29
Grey body, red eyes, vestigial wings, long bristles	1
Grey body, red eyes, vestigial wings, short bristles	3

Grey body, purple eyes, normal wings, long bristles	60
Grey body, purple eyes, normal wings, short bristles	61
Grey body, purple eyes, vestigial wings, long bristles	412
Grey body, purple eyes, vestigial wings, short bristles	407
Total	2002

The four genes on the chromosome are illustrated in Figure 1. While the diagram is not to scale and the genes are not labelled, you know that genes A and B are closer to each other than genes B and C are.

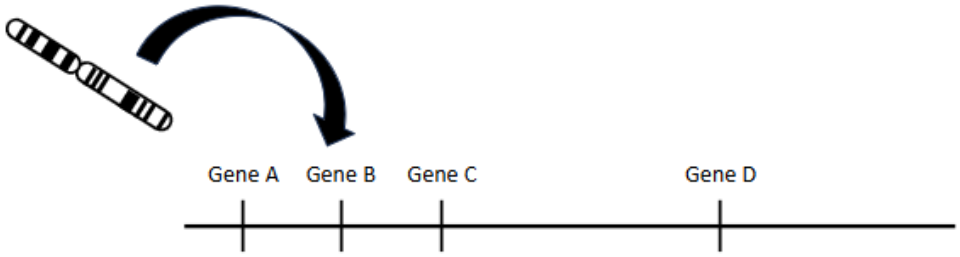


Figure 1: Diagram of genes on chromosome. Not to scale.

Q1. Gene D is so far apart from the other three gene loci that it is practically genetically unlinked. Which gene does gene D represent? **(10 points)**
(Select the correct option.)

A. b^+/b
B. pr^+/pr
C. vg^+/vg
D. s^+/s

Q2. The other 3 genes are genetically linked to each other. Which gene does gene B represent? **(10 points)**
(Select the correct option.)

A. b^+/b
B. pr^+/pr
C. vg^+/vg
D. s^+/s

Q3. Calculate the genetic distance between genes A and B in map units (m.u.). **(20 points)**
(Enter your answer correct to 3 s.f. Do not enter any units.)

Q4. Calculate the genetic distance between genes B and C in map units (m.u.). **(20 points)**
(Enter your answer correct to 3 s.f. Do not enter any units.)

For a general heterozygote with genotype *AaBbCc*, where the gene loci *A/a*, *B/b* and *C/c* lie on the same chromosome in that order, two crossover events must occur to get a gamete with genotype *aBc* or *AbC*, which are known as double recombinants (Figure 2). Hypothetically, the expected frequency of double recombinants can be derived from multiplying the genetic distance between *A/a* and *B/b* and the genetic distance between *B/b* and *C/*c. However, in reality, the observed frequency of double recombinants is often lower due to a phenomenon known as crossover interference. The ratio of the observed frequency of double recombinants to the expected frequency is known as the coefficient of coincidence. The complement of the coefficient of coincidence is the degree of interference.

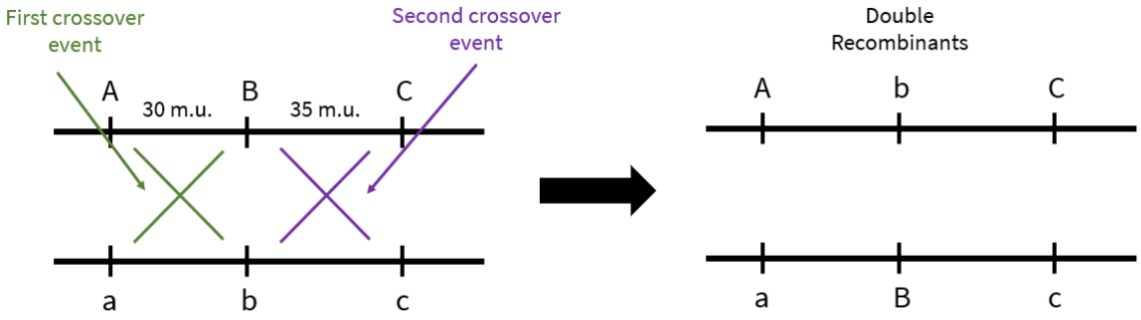


Figure 2: Double recombinants

Q5. Calculate the degree of interference observed. **(30 points)**
(Enter your answer correct to 3 s.f.)

Q6. Dr Pahari ran out of *Drosophila melanogaster* with genotype $bbprprvgvgss$. He decides to cross 2 *Drosophila melanogaster* heterozygotes from the F_1 generation to create more *Drosophila melanogaster* with genotype $bbprprvgvgss$. What is the chance that an offspring fly has the desired genotype? **(40 points)**
(Enter your answer as a decimal correct to 3 s.f.)

Answers and Explanations

Q1.

Answer: **D**

Explanation: The gene that is unlinked is inherited independently from the other genes. Hence, the parental genotype *AaBbCcDd*: recombinant genotype *AaBbCcdd* = 1:1. This is seen for bristle length, so gene D is s^+/s.

Q2.

Answer: **B**

Explanation: Compared to the parental genotype, the recombinant genotype *AabbCcDd* will have a far lower frequency than the parental genotype *AaBbCcDd* as 2 crossover events have to occur. This is seen for eye colour so gene B is pr^+/pr.

Q3.

Answer: **6.14**

Explanation:

$$\text{Genetic distance} \;=\; \text{Recombination frequency} = \frac{Number\ of\ recombinants}{Total\ number\ of\ offspring}$$

$$Genetic\ distance = \frac{2+2+30+28+28+29+1+3}{2002} \times 100 = 6.1439 = 6.14\ mu$$

Q4.

Answer: **12.4**

Explanation: Similarly:

$$Genetic\ distance = \frac{61+58+2+2+1+3+60+61}{2002} * 100 = 12.388 = 12.4\ mu$$

Q5.

Answer: **0.475 or 0.467**

$$Frequency = 2002 * 0.061439 * 0.12388 = 15.237$$

$$Interference = 1 - 8/15.237 = 0.475$$

If you used the 3 s.f. answers instead of the 5 s.f. answer, you would get the answer below. Both answers were accepted.

$$Frequency = 2002 * 0.0614 * 0.124 = 15$$

$$Interference = 1 - 8/15 = 0.467$$

Q6.

Answer: **0.0423**

Explanation: This is a double heterozygous cross, but in this case, 3 genes are linked together. First, the chance that both recessive alleles for bristles are inherited together is $\frac{1}{4}$. Then, the chance that the chromosomes with the recessive alleles are inherited together is $\frac{1}{4}$. Next, the chance that there is no crossover between the b^+/b and pr^+/pr loci for *both* chromosomes is $(1 - 0.0614)^2$ and the chance that there is no crossover between the pr^+/pr and s^+/s loci for *both* chromosomes is $(1 - 0.124)^2$.

Hence:

$$Frequency = \frac{1}{16}(1 - 0.0614)^2(1 - 0.124)^2 = 0.0423$$

P29: Y2H? Y2K?

(190 points)

The Yeast-Two-Hybrid (Y2H) System is a molecular biology technique used to discover protein-protein interactions. Y2H also makes use of transcription factors and the yeast *Saccharomyces cerevisiae* (*S. cerevisiae)*.

All transcription factors are made of two modular domains: a DNA-binding domain (BD) and an activation domain (AD) that recruits RNA polymerase to bind to it. Hence the transcription factor (TF) is necessary as RNA polymerase cannot bind to the promoter by itself, and a transcription factor that lacks either domain is non-functional.

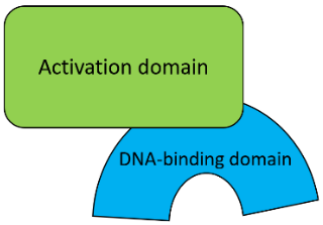


Figure 1: BD and AD of Transcription Factors

A reporter gene such as *lacZ* with a promoter is chosen. Using recombinant technology, the genes for the two proteins of interests, say X and Y, are fused to the genes coding for AD and BD of the transcription factor respectively. The AD and BD-coding genes are separated, often on different plasmids, so that the two domains are separate and non-functional by itself. The two proteins of interest, say X and Y, are fused to AD and BD respectively, forming two proteins AD-X (prey) and Y-BD (bait). If the two proteins interact, X and Y will bind with each other, forming an AD-X-Y-BD protein complex which brings AD and BD close to each other restoring the TF, thus the TF is now functional and transcription of the reporter gene will occur. Transcription for the reporter gene *lacZ* can be seen by the products of the gene. In this case, *lacZ* produces β-galactosidase which hydrolyses X-gal to form a blue product. Conversely, no product will be formed if the two proteins do not interact as the TF cannot be restored.

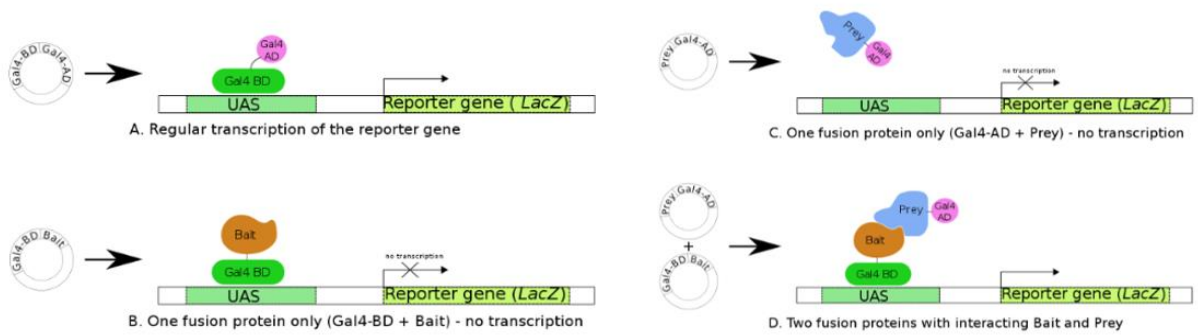


Figure 2: Bait and Prey on Y2H

Faith is investigating the interactions among 5 proteins, P1, P2, P3, X, and Y. X is known to interact with Y. The figure below shows the growth and β-galactosidase activity of yeast cells expressing different combinations of BD and AD fusions. The reporter gene codes for enzymes that synthesise leucine as well as β-galactosidase. The combinations of the plasmids are shown in Figure 3.

Strain	Prey + Bait
1	AD-X + BD-Y
2	AD-P1 + BD-P2
3	AD-P2 + BD-P1
4	AD-P2 + BD-P3
5	AD-P3 + BD-P2
6	AD-P1 + BD-P3

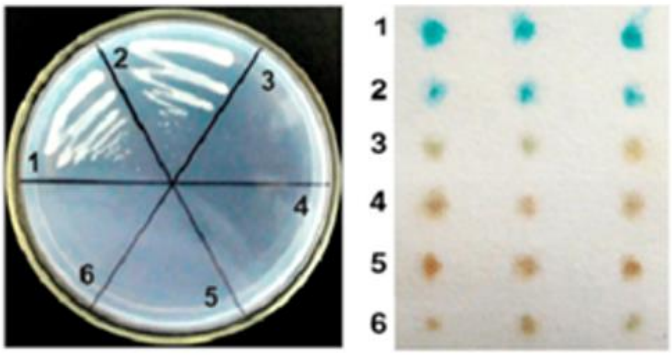


Figure 3: Y2H Assay Results on the five proteins. **Left panel:** Prey and Bait in yeast strains. **Middle panel:** Growth of yeast cells on a SD/-Leu dropout plate. **Right panel:** Activity of the β-gal of indicated strains.

Q1. Answer the following questions regarding the strains and the proteins. **(30 points)**
(Enter the correct answer to each row. Use None, 1, 2, 3, 4, 5, 6, or All to represent the strains, and P1, P2, or P3 to represent proteins.)

Question	**Strain/Proteins**
Positive control	
Negative control	
Which are the two proteins that bind together? Leave your answer as “P1P2” or “P2P3” or “P1P3”.	

Q2. Indicate whether the following statements regarding modifications to the Y2H assay are true or false. “Work” implies that the assay is accurate and valid and the data can be used, but not necessarily yielding a positive result. **(40 points)**
(Mark each statement as true or false.)

A. Y2H can still work even if the proteins are not folded in their specific conformation as long as the amino acid sequence where they interact is present.
B. Y2H can still work between a soluble protein and an insoluble protein like myosin.
C. Y2H can still work even if the two fusion proteins can independently activate the reporter gene.
D. The Y2H system established in Figure 3 will still work even if the β-galactosidase enzyme coded for is non-functional.

Q3. Indicate whether the following statements regarding the Y2H assay are true or false. **(40 points)**

(Mark each statement as true or false.)

A. A Y2H assay with RNA polymerase as bait and another protein as prey yields a blue solution. This means that RNA polymerase and the protein definitely interact in human cells.
B. As the strain of yeast used cannot carry out oxidation between cysteine residues, a Y2H assay involving insulin and RTK will yield a negative result.
C. Adding a substance that increases the electrostatic attraction between the two proteins of interest can increase the specificity of Y2H.
D. Y2H can be used to discover most of the substrates that enzymes bind to.

Faith is interested in investigating the interactions between seven proteins (A to G) in humans. She makes use of a Y2H assay to deduce the interactions between different proteins.

Figure 4 shows the results of Y2H with X-gal using the different proteins as bait and prey. Unfortunately, due to poor experimental techniques, some of Faith's results are invalid and appear as black on Figure 4. Fortunately, Faith knows the Protein E is the first precursor in this pathway.

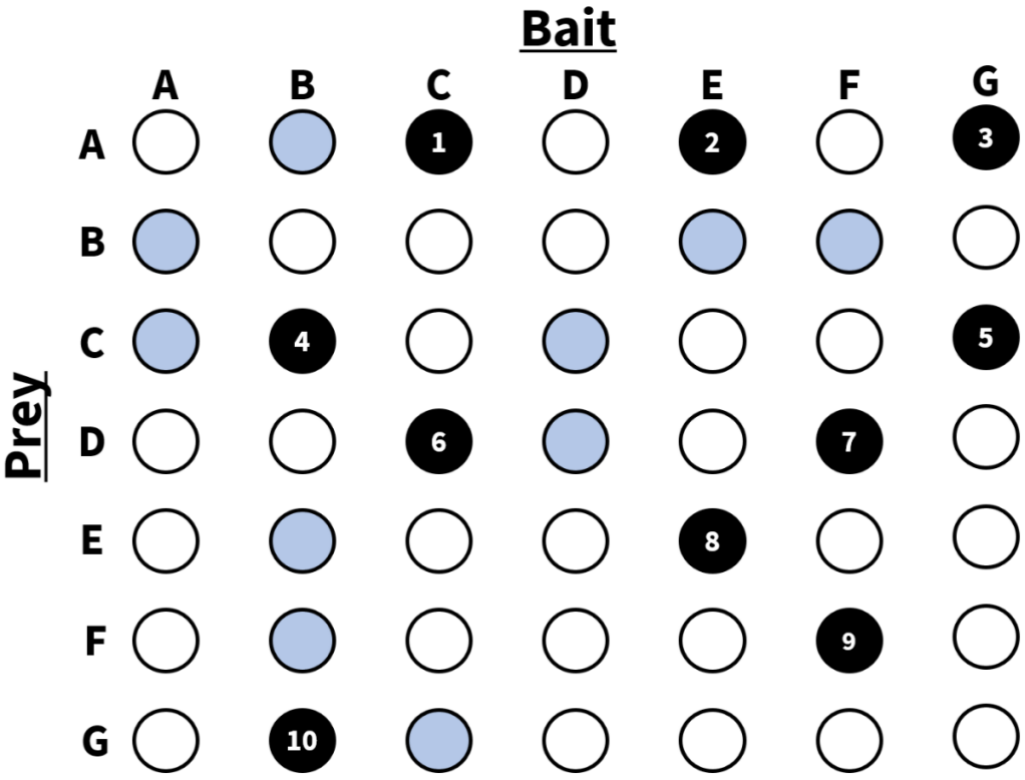


Figure 4: Results of Y2H using different proteins as bait and prey. Black dots represent failed Y2H assays due to poor experimental techniques. The failed assays are numbered from 1 to 10.

Q4. Which protein(s) act downstream to protein A? **(10 points)**

(Select all correct options.)

A. None
B. Protein B
C. Protein C
D. Protein D
E. Protein E
F. Protein F
G. Protein G

Q5. Based on Figure 4, which protein most likely exists as a homodimer? **(10 points)**

(Select the correct option.)

A. Protein A
B. Protein B
C. Protein C
D. Protein D
E. Protein E
F. Protein F
G. Protein G

Q6. Based on Figure 4, indicate which of the failed assays would definitely have been positive (blue) if the assay had worked properly. **(10 points)**

(Select all correct options.)

A. 1
B. 2
C. 3
D. 4
E. 5
F. 6
G. 7
H. 8
I. 9
J. 10
K. None

Q7. By deducing the interaction pathway between these seven proteins, indicate whether the following statements are true or false. **(50 points)**

(Mark each statement as true or false.)

A. Protein A acts downstream of Protein B.
B. Protein B acts downstream of Protein G.
C. Protein D acts upstream of Protein G.
D. Protein D acts downstream of Protein B.
E. Protein F acts downstream of Protein D.

Answers and Explanations

Q1.

Answer: **1, None, P1P2**

Explanation: Yeast growth is only seen in strains 1 and 2. Hence, there are only interactions in strain 1 and 2, allowing AD and BD to come close to interact and transcribe β-galactosidase. β-galactosidase then cleaves X-gal to produce the blue product. Enzymes that synthesise leucine are also transcribed allowing the yeast strains to survive on the medium. X and Y are known to interact, so they are used as the positive control to observe the positive result when the proteins interact. The positive control experiment is thus strain 1. There is no negative control; a negative control would include either two proteins that are known to not interact or AD and BD not bound to any protein to observe whether they intrinsically interact and thus affecting the results. Strain 2 shows interactions between AD and BD thus implying P1 and P2 interact. While strain 3 shows no interactions, this could be due to the direction of interaction between the two proteins.

Q2.

Answer: **FFFT**

Explanation:

A. Y2H relies on the interactions between proteins. Proteins interact due to their specific 3D conformation, hence the loss of the conformation will affect interactions and thus yield an inaccurate result.
B. An insoluble protein will not be able to dissolve in the intracellular cytosol for interactions.
C. If they can independently activate the reporter gene, the reporter gene will be activated regardless of any interactions between the proteins, thus causing false positives.
D. If the reporter gene is transcribed, the non-functional β-galactosidase will be unable to digest X-gal to produce the blue product. However, the yeast will still be able to grow in the middle panel because the reporter gene also codes for enzymes which synthesises leucine. Therefore, the results can still be seen.

Q3.

Answer: **FTFF**

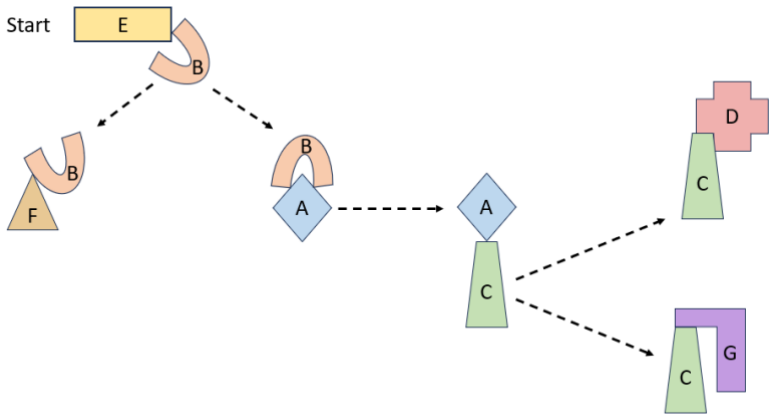
Explanation:

A. This may be a false positive as they may interact in the yeast cells but may not interact in human cells as they may be spatially separated.
B. The precursor insulin polypeptide required three disulfide bridges for it to fold to its correct conformation to bind to RTK. Since this is not present in the yeast, the insulin will be unable to bind to RTK, hence yielding a false negative result.
C. Increasing the electrostatic attraction increases the likelihood that any interactions between the proteins are due to the intrinsic attraction between them rather than the fact that they interact with each other as their conformation is complementary. Hence, this can yield false positives and is thus less specific.
D. Many substrates of proteins are not proteins. Y2H only investigates protein-protein interactions.

Deducing the interaction pathway.

Interactions between the bait and prey will yield a positive blue result. In addition, it does not matter if the protein is the bait or prey; we can assume that they will yield a positive blue result regardless whether they are the bait or prey. The first precursor is E, so we observe which proteins interact with E. Only B interacts with E, hence B must be the next step of the pathway. Next, B interacts with A and F too, so the pathway bifurcates to A and F. A interacts with C, so C comes downstream of A. C interacts with D and G, so there is another bifurcation. D and G do not interact with any other proteins, so we stop the pathway here. Moving on to F, F does not interact with any other proteins, so the pathway stops here too.

Hence the pathway can be seen as:



Q4.

Answer: **C, D, G**

Explanation: It is clear from the pathway diagram above.

Q5.

Answer: **D**

Explanation: When D is both the bait and prey, a positive blue result is seen, implying that they likely interact to form a homodimer. Although the result of E and F when they are both bait and prey are not seen, we do not have sufficient evidence to conclude they will form homodimers, so the only answer is D.

Q6.

Answer: **A, E, F**

Explanation: By forming a reflection about the imaginary diagonal line cutting from A/A to G/G, we can observe that if, say A is the bait and B is the prey and they interact, then if their roles are reversed as in A is the prey and B is the bait, they will also interact and form a positive result.

Q7.

Answer: **TFFTF**

Explanation: It is trivial after looking at the pathway diagram above. Protein B acts upstream of Protein G thus statement B is false. Protein D is not in the same pathway as Protein G, and Protein F is not in the same pathway as Protein D, so we cannot conclude their upstream and downstream relationships.

Credits

Figure 2: Modified from Anna. (2007, October). *Two hybrid assay.* Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Two_hybrid_assay.svg

Figure 3: Modified from Lin, Y., Li, Y., Zhu, Y., Zhang, J., Li, Y., Liu, X., Jiang, W., Yu, S., You, X.-F., Xiao, C., Hong, B., Wang, Y., Jiang, J.-D., & Si, S. (2012). Identification of antituberculosis agents that target ribosomal protein interactions using a yeast two-hybrid system. *Proceedings of the National Academy of Sciences*, *109*(43), 17412–17417. https://doi.org/10.1073/pnas.1110271109

P30: The Animal Kingdom

(220 points)

The Lee Kong Chian National History Museum (LKCNHM) is located in the Kent Ridge campus of the National University of Singapore and boasts over a million specimens from across Southeast Asia. On a learning journey to the LKCNHM, Kelly was very fascinated by the large biodiversity of specimens there. She asked the docent, "What's the theme? There's always a theme." The docent replied, "I don't tell you the theme, you see the theme," and he promptly handed her an information booklet with a phylogenetic tree. The docent continued, "Here, each number in the dichotomous key corresponds to an animal specimen in the museum. See if you can identify them."

Dichotomous Key

1. Radial symmetry
    1. Yes — go question 2
    2. No — go to question 3
2. Nematocysts
    1. Yes – Species 2
    2. No – Species 6
3. Vertebral Column
    1. Yes — go to question 5
    2. No — go to question 4
4. Sessile adult form
    1. Yes – Species 9
    2. No – Species 12
5. Bones
    1. Yes – go to question 7
    2. No – go to question 6
6. Jaw
    1. Yes – Species 1
    2. No – Species 3
7. Amniote
    1. Yes – go to question 10
    2. No – go to question 8
8. Positive breathing pressure
    1. Yes – Species 13
    2. No – go to question 9
9. Lobed fin
    1. Yes – Species 10
    2. No – Species 4
10. Temporal opening on skull
    1. Yes – go to question 11
    2. No – Species 5
11. Egg laying
    1. Yes – go to question 13
    2. No – go to question 12
12. The species have transmissible Cancer
    1. Yes – Species 11
    2. No – Question 15
13. Eggshell hardness
    1. Soft – Question 14
    2. Hard – Species 15
14. Lactation
    1. Yes – Species 14
    2. No – Species 7
15. Presence of epipubic bones?
    1. Yes – Species 8
    2. No – Species 16

Figure 1: Kelly's Dichotomous Key

Kelly's friend, Ryan, looked really interested in her dichotomous key. Hence, the docent also passed him an information booklet, but this booklet instead contained two phylogenetic trees. The docent pointed out a board in the far distance, saying, "Look at that board. Those 16 animal specimens are each represented by a number on Kelly's dichotomous key and a letter on Ryan's phylogenetic trees. But some of the animal specimens are already filled in in the phylogenetic trees for you!"

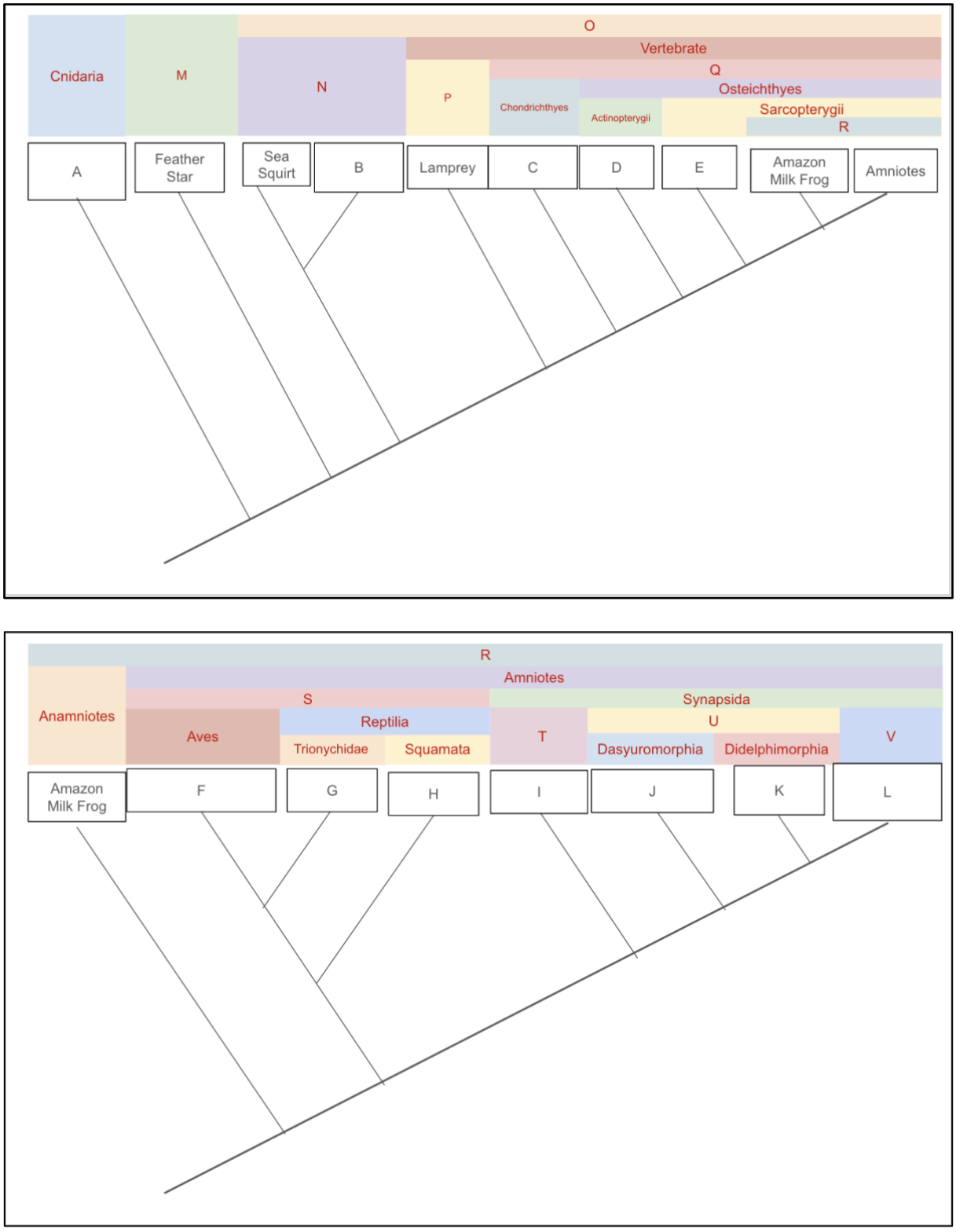


Figure 2: Ryan's two phylogenetic trees

Let's help Kelly and Ryan match the animal specimens to their corresponding numbers and letters.

Q1. Match the following animal specimens to the number and letter they represent. Enter your answer by **entering the corresponding number and then the corresponding letter to each row**. Do not leave any space. For example, if your answer is Animal 5 and Animal A, enter *5A*. **(30 points)**

(Enter a number and then a letter to each row.)

Animal Specimen	**Number (1-16) + Letter (A-L)**
Crescent-caped lophorina	
Pacific sea nettle	
Opossums	

Q2. Match the following animal specimens to the number and letter they represent. Enter your answer by **entering the corresponding number and then the corresponding letter to each row**. Do not leave any space. For example, if your answer is Animal 5 and Animal A, enter *5A*. **(30 points)**

(Enter a number and then a letter to each row.)

Animal Specimen	**Number (1-16) + Letter (A-L)**
Echidna	
Softshell turtles	
Greenland shark	

Q3. Match the following animal specimens to the number and letter they represent. Enter your answer by **entering the corresponding number and then the corresponding letter to each row**. Do not leave any space. For example, if your answer is Animal 5 and Animal A, enter *5A*. **(30 points)**

(Enter a number and then a letter to each row.)

Animal Specimen	**Number (1-16) + Letter (A-L)**
Lungfish	
Slowworms	
Giant Larvacean	

Q4. Match the following animal specimens to the number and letter they represent. Enter your answer by **entering the corresponding number and then the corresponding letter to each row**. Do not leave any space. For example, if your answer is Animal 5 and Animal A, enter *5A*. **(30 points)**

(Enter a number and then a letter to each row.)

Animal Specimen	**Number (1-16) + Letter (A-L)**
Sunfish	
Tasmanian Devil	
Asian Small-Clawed Otters	

On the next page of his information booklet, Ryan noticed several keywords (Figure 3) that he presumes matches to the letters above the phylogenetic trees.

Chordata	Sauropsida	Gnathostomes	Tetrapoda	Echinodermata
Monotremata	Eutheria	Marsupialia	Cyclostomes	Tunicata

Figure 3: Keywords

Q5. Match each letter on the top of the phylogenetic trees with their corresponding keywords. **(50 points)**

(Enter a word from Figure 3 to each row.)

Letter	**Keyword**
M	
N	
O	
P	
Q	

Q6. Match each letter on the top of the phylogenetic trees with their corresponding keywords. **(50 points)**

(Enter a word from Figure 3 to each row.)

Letter	**Keyword**
R	
S	
T	
U	
V	

Answers and Explanations

Analysing the Dichotomous Key

*While **Q1** to **Q4** were separate questions, the dichotomous key should be approached holistically to solve all 4 questions at once. The animals were separated into different questions to prevent participants from losing too many marks over a few wrong matches.*

1. Only cnidarians and echinoderms exhibit some form of radial symmetry
2. Only cnidarians such as **Pacific sea nettle (species 2A)** possess nematocysts, which are stinging cells. Echinoderms such as feather stars (species 6) do not.
3. To separate the vertebrates from the invertebrates (sea squirt and giant larvaceans, which are tunicates and therefore invertebrates. They do, however, possess a notochord).
4. Sea squirts (species 9) are sessile in their adult form while **giant larvaceans (species 12B)** remain motile throughout their lives
5. To separate the animals with bones from those without (lampreys and sharks, the latter of which have only cartilaginous skeletons).
6. A defining trait of lampreys (species 3) is that they lack a jaw. **Greenland sharks (species 1C)** on the other hand, do possess a jaw.
7. Amniotes include the sauropsids (reptiles and birds) and the synapsids (of which only mammals remain today), defined by their 3 extra-embryonic membranes: amnion, chorion, and allantois. Frogs and lungfishes are anamniotic, which is why they lay their eggs in water to prevent them from drying out.
8. Frogs such as the Amazon Milk Frog (species 13) are notable for their positive breathing pressure, where they actively suck air into their lungs to breathe. On the other hand, other animals such as birds and mammals have negative breathing pressures, where they expand their ribcage to lower their air pressure in their lungs to draw air in for breathing.
9. The only lobe-finned fish that exists today is the **lungfish (species 10E)**. All other fishes, including **sunfish (species 4D)**, are ray-finned fish. The fins of the former are fleshy, resembling stump-like appendages, whereas fins of the latter have webs of skin covering flexible spines.
10. Anapsids such as **the softshell turtle (species 5G)** have no temporal openings on their skull. Synapsids, which include mammals, have one temporal opening on their skull, and diapsids, which include lizards and birds, have two temporal openings on their skull.
11. Used to differentiate marsupials and eutherians from other animals such as reptiles and monotremes
12. One notable trait of the **Tasmanian devils (species 11J)** is that they have a form of transmissible cancer known as devil facial tumour disease, which is transmitted when one Tasmanian devil bites another.

13. Reptiles and monotremes have eggs with soft shells while birds such as the **crescent-caped lophorina (species 15F)** have eggs with hard shells.
14. Only mammals lactate. Therefore, given that both animals lay soft shell eggs and are not turtles, by elimination, **species 14I**, which lactates, is the **echidna**, and **species 7H**, which does not lactate while still producing soft-shell eggs, is the **slowworm**, a type of legless lizard.
15. Marsupials such as **opossums (species 8K)** as well as monotremes have epipubic bones, while modern placental animals such as **asian small-clawed otters (species 16L)** lack them.

Q1.

Answer: **15F, 2A, 8K**

Q2.

Answer: **14I, 5G, 1C**

Q3.

Answer: **10E, 7H, 12B**

Q4.

Answer: **4D, 11J, 16L**

Q5.

Answer: **Echinodermata, Tunicata, Chordata, Cyclostomes, Gnathostomes**

Explanation:

A. As feather stars are members of the phylum Echinodermata.
B. As both sea squirts and giant larvaceans belong in the subphylum Tunicata.
C. All fishes, including jawless fishes (cyclostomes, the only existing superclass of the infraphylum of jawless fishes agnatha), jawed vertebrates (gnathostomes), as well as tunicates, belong to the phylum Chordata.
D. Cyclostome is the only extant clade of jawless fishes, including lampreys.

E. All jawed vertebrates such as cartilaginous fishes, bony fishes, amphibians and amniotes belong to **Gnathostome**.

Q6.

Answer: **Tetrapoda, Sauropsida, Monotremata, Marsupialia, Eutheria**

Explanation:

A. All 4-limbed vertebrate animals, as well as their descendants such as snakes, legless lizards and cetaceans, belong to the superclass **Tetrapoda**.
B. **Sauropsida** is the Clade encompassing all modern reptiles and birds, which are technically descendants of theropod dinosaurs and are more closely related to crocodilians than turtles and lizards.
C. The echidna are one of two extant mammals which lay eggs, which puts them in the order **Monotremata.**
D. Opossums are **marsupials,** being the only marsupials that inhabit North America. Their most notable difference from eutherians is that they give birth to their young prematurely, with many female marsupials having a front pouch to carry said premature offspring.
E. **Eutherians** include most extant mammals, including humans, asian small-clawed otters, cats, dolphins, etc. All eutherians lack epipubic bones, which allow for the expansion of the abdomen during pregnancy. Placental mammals are the only extant group representing the eutherians, characterised by the fact that they carry their young in the mothers' uterus until a relatively late stage in their development.

Credits

Content reference:

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P31: Vanda Miss Joaquim

(150 points)

While drinking a cup of refreshing sugarcane juice, you stroll in the Singapore Botanic Gardens. The Singapore Botanic Gardens was founded in 1859 and is one of only three gardens in the world to be honoured as a UNESCO World Heritage Site. As you were walking, you stumbled into your friend Lionel who works there with NParks. You notice that he looks flustered. Maybe he needs a cup of sugarcane juice!

Upon asking him, Lionel reveals that he works in the Orchid Garden and needs help with one of his orchid plants.

Figure 1: Singapore National Orchid Garden

Singapore's national flower is an orchid called Vanda Miss Joaquim (Figure 2). *(You may have noticed the orchid on the SBL website too!)* Vanda Miss Joaquim was selected as the national flower on 15 April 1981 and was created by horticulturist Agnes Joaquim by crossing two orchid species, *Vanda teres* and *Vanda hookeriana*, in her garden. The orchid was named after her for her contribution to the creation of this flower. However, recent scientific research has revealed that these two orchid species are actually of the *Papilionanthe* genus, and thus the scientific name for Vanda Miss Joaquim has been changed to *Papilionanthe Miss Joaquim*. Nonetheless, the flower is still commonly called Vanda Miss Joaquim.

Figure 2: Vanda Miss Joaquim

Most commercial orchids are tetraploids, and so are these hybrids. During meiosis, each of the four homologous chromosomes associate randomly with each other forming two homologue pairs. Hence, each daughter cell receives two of the four homologues. Orchids are the most diverse family of Angiosperms and belong to the family Orchidaceae. They have colourful blooms and fragrances to attract insects for pollination. However, orchids are not immune to pests, and often are infested by aphids and caterpillars.

He recently discovered that one of the *Arachnis hookeriana* orchid plants in the Singapore Botanic Gardens was immune to aphids. Upon investigation, he discovered a dominant gene *A* in the plant which confers resistance to aphids by secreting neem oil which repels aphids. This plant has the genotype *AAaa* at its gene locus. Lionel also discovered that while most of the *A. hookeriana* plants were *aaaa*, some of the neighbouring *A. hookeriana* plants have the genotype *Aaaa* but do not have any resistance to aphids. He hence deduces that at least two dominant *A* alleles are required for resistance.

Lionel intends to select for this gene *A* by crossing different *A. hookeriana* plants together. He does so by first placing a toothpick under the anther to extract the pollen from one plant, then pushing the pollen into the stigma of the other plant.

Q1. Lionel crossed several orchid plants together based on the table below. Crossing of the parents produces the F1 (first filial) generation. The F1 generation is then crossed with itself to obtain the F2 generation and so on. Indicate the earliest generation that he can obtain an *AAAA* plant in the following crosses. If you think that it can never be obtained, leave your answer as *-1*. **(40 points)**
(Enter the correct answer to each row. Leave your answer as the filial generation in short form i.e. F1, or F2, or F3…)

Cross	**Earliest generation where *AAAA* can be obtained**
Aaaa self-cross	
AAaa self-cross	
Aaaa x *aaaa*	
AAaa x *Aaaa*	

Q2. If Lionel were to test cross a *A. hookeriana* plant with genotype *AAaa*, what is the probability of obtaining an *Aaaa* offspring plant in the F1 generation? **(20 points)**
(Enter your answer as a decimal correct to 3 s.f.)

Q3. Lionel then decided to cross a *AAaa* plant with an *Aaaa* plant. What is the probability of getting an offspring plant that is resistant to aphids in the F1 generation? **(30 points)**
(Enter your answer as a decimal correct to 3 s.f.)

Q4. Lionel wishes to obtain pure-bred *AAAA* plants. He randomly picked two aphid-resistant plants from the F1 generation in **Q3** and crossed them. What is the probability of obtaining a pure-bred *AAAA* plant? **(40 points)**

(Enter your answer as a decimal correct to 3 s.f.)

Hybrid orchids can be produced by crossing two different species of orchids together. The pollen of one species of orchids is transferred to the stigma of another species of orchid, producing a hybrid of both species. Fertilisation occurs in a similar manner to other angiosperms. The pollen grain contains a generative cell and a tube cell, the latter of which grows into the style leading the way for the generative cell. The generative cell divides by mitosis forming two sperms. As the pollen tube enters the ovule through the micropyle, one sperm fertilises the egg situated between the synergids while the other fertilises the polar nuclei. The polar nuclei are each formed when the meiotic product in the female plant undergoes mitosis to produce two polar nuclei. The embryo then germinates after imbibition to form an orchid hybrid plant.

While most orchids are either diploids or tetraploids, there have been reports of extremely rare cases of orchids with higher ploidy levels. Lionel artificially bred two species of orchid with higher ploidy levels by inducing non-disjunction using colchicine. One species of the orchid is decaploid $(10n)$ while the other is an octoploid $(8n)$.

Q5. Lionel transferred the pollen grain from the decaploid $(10n)$ species to the stigma of the octoploid species of orchid $(8n)$. Indicate the ploidy of the resultant endosperm and embryo as a multiple of n. **(20 points)**

(Enter your answer to each row as a multiple of n. For example, if you think that it is diploid, leave your answer as 2n. If you think that the ploidy level is a decimal, leave your answer to 1 d.p. like 3.5n. If you think there are extra copies of a chromosome, leave your answer as the sum of a multiple of n and an integer. For example, if you think there is trisomy 21 in a diploid, leave your answer as 2n + 1.)

Tissue	**Ploidy (n)**
Endosperm	
Embryo	

Answers and Explanations

It is important to take note of some points in the preamble:

“During meiosis, each of the four homologous chromosomes associate randomly with each other forming two homologue pairs. Hence, each daughter cell receives two of the four homologues.” This states that the gametes will receive two homologues instead of the usual one in diploids. This is useful for **Q1**, **Q2**, **Q3**, **Q4**.

“At least two dominant A alleles are required for resistance.” This is important as it deviates from the usual phenomenon where one dominant allele is sufficient to indue the dominant phenotype. This is useful for **Q3** and **Q4**.

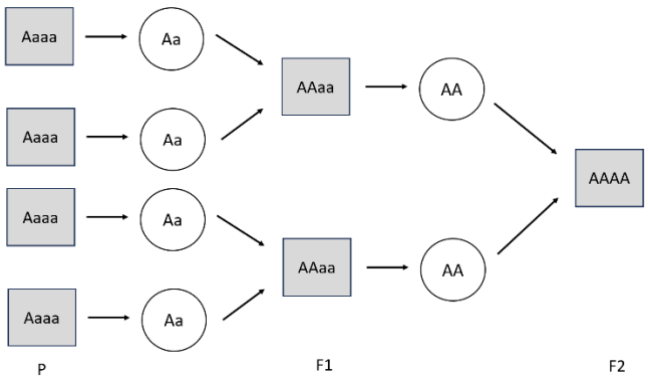
“He does so by first placing a toothpick under the anther to extract the pollen from one plant, then pushing the pollen into the stigma of the other plant.” This method of crossing to form hybrids is useful for **Q5**.

Q1.

Answer: **F2, F1, F3, F2**

Explanation:

A. To obtain *AAAA* the earliest, both gametes that give rise to the F1 generation must contain the *A* allele and hence both gametes must be *Aa*. The F1 genotype will thus be *AAaa*. Hence, *AAAA* can be obtained in the F2 generation if both gametes are *AA*.



B. This is just one step ahead of the above scenario. *AAAA* can be obtained in the F1 generation if both gametes are *AA*.
C. In this cross, the F1 generation containing *A* will be *Aaaa*. This is the same as scenario A, so we need one extra generation as compared to scenario A. Hence the answer is F3.
D. The F1 generation can have 3 *A* alleles with two from one parent and one from another parent. Thus, the parents of the F2 generation can each pass down two *A* alleles, forming the *AAAA* orchid.

Q2.

Answer: **0.667**

Explanation: The ratio of the gametes that are produced by *AAaa* is:

$$\mathbf{1}\ \boldsymbol{AA} : \mathbf{4}\ \boldsymbol{Aa} : \mathbf{1}\ \boldsymbol{aa}$$

Since the other parent plant is *aaaa*, the required probability is the probability of obtaining a *Aa* gamete, so the probability is $\frac{\mathbf{4}}{\mathbf{6}} = \frac{\mathbf{2}}{\mathbf{3}}$.

Q3.

Answer: **0.500**

Explanation: As stated above, the ratio of the gametes that are produced by *AAaa* is:

$$\mathbf{1}\ \boldsymbol{AA} : \mathbf{4}\ \boldsymbol{Aa} : \mathbf{1}\ \boldsymbol{aa}$$

The ratio of the gametes that are produced by *Aaaa* is:

$$\mathbf{1}\ \boldsymbol{Aa} : \mathbf{1}\ \boldsymbol{aa}$$

To obtain an aphid-resistant plant, 2 *A* alleles must be present. There are two possible ways to obtain this.

Case 1: *AA* gamete obtained from parent plant

$$Probability = P(AA) \times P(any\ gamete) = \frac{1}{6} \times 1 = \frac{1}{6}$$

Case 2: *Aa* gamete obtained from both plants

$$Probability = P(Aa) \times P(Aa) = \frac{4}{6} \times \frac{1}{2} = \frac{1}{3}$$

Hence the total probability is: $Probability = \frac{1}{6} + \frac{1}{3} = \frac{1}{2}$.

Q4.

Answer: **0.494**

Explanation: We first look at the genotypic ratio of the F1 generation in **Q3**.

By simple crossing of each possibility, we can tell that the ratio is:

$$\mathbf{1}\ \boldsymbol{AAAa} : \mathbf{1}\ \boldsymbol{AAaa} : \mathbf{4}\ \boldsymbol{AAaa} : \mathbf{4}\ \boldsymbol{Aaaa} : \mathbf{1}\ \boldsymbol{Aaaa} : \mathbf{1}\ \boldsymbol{aaaa}$$

Which simplifies to:

$$\mathbf{1}\ \boldsymbol{AAAa} : \mathbf{5}\ \boldsymbol{AAaa} : \mathbf{1}\ \boldsymbol{Aaaa} : \mathbf{1}\ \boldsymbol{aaaa}$$

Since **only aphid-resistant** plants were selected, we only look at the ones with at least 2 *A* alleles. Hence, the ratio is simply: $\mathbf{1}\ \boldsymbol{AAAa} : \mathbf{5}\ \boldsymbol{AAaa}$.

There are three possible crosses which we will analyse individually:

Case 1: *AAAa x AAAa*

The ratio of the gametes that are produced by *AAAa* is:

$$\mathbf{1}\ \boldsymbol{AA} : \mathbf{1}\ \boldsymbol{Aa}$$

Hence, the probability of getting *AAAA* by obtaining *AA* from both parents is:

$$\boldsymbol{Probability} = \frac{\mathbf{1}}{\mathbf{2}} \times \frac{\mathbf{1}}{\mathbf{2}} = \frac{\mathbf{1}}{\mathbf{4}}$$

The probability that such a cross occurs (based on the ratios of the parents) and that *AAAA* is obtained is thus:

$$\boldsymbol{Probability} = \frac{\mathbf{1}}{\mathbf{6}} \times \frac{\mathbf{1}}{\mathbf{6}} \times \frac{\mathbf{1}}{\mathbf{4}} = \frac{\mathbf{1}}{\mathbf{144}}$$

This is essentially asking yourself: 1. What is the probability that I pick the *AAAa* plant, then the probability that I pick another *AAAa* plant, and then the probability that after crossing them, I get *AAAA* offspring?

Of course, we need to operate under the assumption that the population of F1 plants is so large that picking a plant does not affect the chances of the genotype of the net plant picked.

Case 2: *AAaa x AAaa*

As stated above, the ratio of the gametes that are produced by *AAaa* is:

$$\mathbf{1}\ \boldsymbol{AA} : \mathbf{4}\ \boldsymbol{Aa} : \mathbf{1}\ \boldsymbol{aa}$$

Hence, the probability of getting *AAAA* by obtaining *AA* from both parents is:

$$\boldsymbol{Probability} = \frac{\mathbf{1}}{\mathbf{6}} \times \frac{\mathbf{1}}{\mathbf{6}} = \frac{\mathbf{1}}{\mathbf{36}}$$

The probability that such a cross occurs (based on the ratios of the parents) and that *AAAA* is obtained is thus:

$$\boldsymbol{Probability} = \frac{\mathbf{5}}{\mathbf{6}} \times \frac{\mathbf{5}}{\mathbf{6}} \times \frac{\mathbf{1}}{\mathbf{36}} = \frac{\mathbf{25}}{\mathbf{1296}}$$

Case 3: *AAAa x AAaa*

The probability of getting *AAAA* by obtaining *AA* from both parents is:

$$\boldsymbol{Probability} = \frac{\mathbf{1}}{\mathbf{6}} \times \frac{\mathbf{1}}{\mathbf{2}} = \frac{\mathbf{1}}{\mathbf{12}}$$

The probability that such a cross occurs (based on the ratios of the parents) and that *AAAA* is obtained is thus:

$$\boldsymbol{Probability} = \frac{\mathbf{5}}{\mathbf{6}} \times \frac{\mathbf{1}}{\mathbf{6}} \times \binom{\mathbf{2}}{\mathbf{1}} \times \frac{\mathbf{1}}{\mathbf{12}} = \frac{\mathbf{5}}{\mathbf{216}}$$

We must multiply by ${}^{2}C_{1}$ as we could have picked the *AAAa* plant followed by the *AAaa* plant, or in the other order.

Hence, the total probability is:

$$\boldsymbol{Probability} = \frac{\mathbf{1}}{\mathbf{144}} + \frac{\mathbf{25}}{\mathbf{1296}} + \frac{\mathbf{5}}{\mathbf{216}} = \frac{\mathbf{4}}{\mathbf{81}}$$

Q5.

Answer: **13n, 9n**

Explanation: It is important to pay attention to the preamble which lists important information regarding double fertilisation in plants.

The sperm in the pollen grain from the decaploid orchid will have a ploidy of 5n due to meiosis. This 5n sperm then fuses with the 4n egg forming a **9n** zygote. The egg has a ploidy of 4n because of meiosis in the octoploid orchid.

During meiosis, the meiotic product with ploidy 4n undergoes mitosis to form two polar nuclei, thus **each** polar nuclei have a ploidy of 4n, so combined together the polar nuclei have a ploidy of 8n. Thus, as the sperm fuses with the polar nuclei and subsequently form the endosperm, the overall ploidy would be **13n**. This should be familiar to participants as diploid angiosperms have a diploid zygote but triploid endosperm due to double fertilisation.

Credits

Figure 1: Gonsalves, T. A. (2023, February 18). *Entrance, National Orchid Garden, Botanic Gardens, Singapore*. Wikipedia. https://en.wikipedia.org/wiki/National_Orchid_Garden#/media/File:Entrance_National_Orchid_Garden_Singapore_Feb23_D72_25450.jpg

Figure 2: *Vanda Miss Joaquim*. Singapore Botanic Gardens. (2024, June 18). https://www.nparks.gov.sg/sbg/our-gardens/tanglin-entrance/vanda-miss-joaquim#:~:text=Vanda%20Miss%20Joaquim%20originated%20from,the%20Singapore%20Botanic%20Gardens%20H.%20N

Content Reference: Arunasalam, S., Ong, E. C., & Fiona, L. (2017). *Vanda Miss Joaquim*. National Library Board Singapore. https://www.nlb.gov.sg/main/article-detail?cmsuuid=a6d4c4a7-18ac-4b8f-a07f-7790d501b8a5#:~:text=Scientific%20research%20has%20also%20revealed,commonly%20called%20Vanda%20Miss%20Joaquim

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Did you know?

Vanda Miss Joaquim and many other orchid hybrids are cultivated in the Singapore Botanic Gardens Orchid Garden which highlights their status as a treasured botanical icon and a source of pride for Singaporeans. In fact, Vanda Miss Joaquim represents a significant part of orchid diplomacy in Singapore. Orchid diplomacy is a gesture of friendship promoting the goodwill between Singapore and other countries. When world leaders visit Singapore in an official capacity, they may receive an orchid hybrid named after them.

The first VIP orchid is the *Aranthera Anne Black* (*Arachnis Maggie Oei* × *Renanthera coccinea*), named after Lady Anne Black in 1956. She was the wife of Robert Black, a former governor of Singapore.

P32: Emma is crying again!

(150 points)

After Emma cried over spilling milk over her data, she promised never to drink milk in the lab again (which she should not have anyway). However, she was quite happy in the lab today as she recently discovered an assay called Electrophoretic Mobility Shift Assay (EMSA) which has a similar name to hers.

Electrophoretic Mobility Shift Assay (EMSA) is an assay to study the interactions of proteins to different segments of DNA, for example with transcription factors. The protein of interest is first added to the radioactively labelled DNA fragments, and then the sample is subjected to electrophoresis. This operates on the principle that DNA fragments bound to proteins will be retarded and thus migrate more slowly than the free linear DNA fragments. Thus, the radioactively labelled DNA can be imaged by radiographic techniques.

Emma needs to determine where the sequence coding for the DNA-binding domain of a newly-discovered transcription factor TFIIN is located in the gene. She constructs five gene constructs from 5' to 3' as seen below and transforms them separately into *E. coli* to form proteins 1 to 5 from each gene construct 1 to 5 respectively as seen in Figure 1.

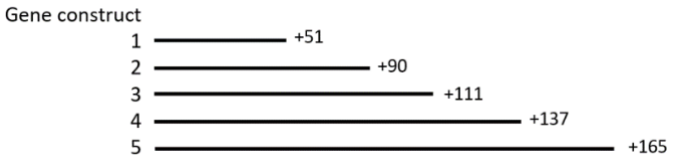


Figure 1: Gene constructs

The transcription factor is known to bind to a 100bp DNSA fragment. The DNA fragment was radioactively labelled, mixed with the various combinations of proteins as seen in the table in Figure 2, and then subjected to an EMSA.

Lane 1	No protein
Lane 2	Protein 1
Lane 3	Protein 2
Lane 4	Protein 3
Lane 5	Protein 4
Lane 6	Protein 5
Lane 7	Protein 1 + 2
Lane 8	Protein 4 + 5

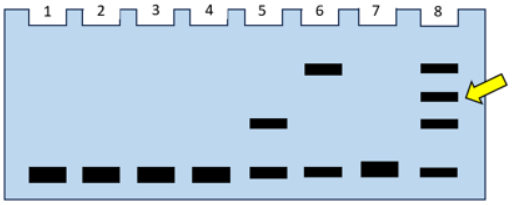


Figure 2: EMSA for TFIIN investigation

Q1. Within which region can the DNA-binding domain of TFIIN be found? **(10 points)**
(Select the correct option.)

A. Upstream of -1
B. -1 to +51
C. +51 to +90
D. +90 to +111
E. +111 to +137
F. +137 to +165
G. Downstream of +165
H. Cannot be deduced

Q2. An additional band can be seen in Lane 8 as indicated by the yellow arrow. Emma made several hypotheses regarding its formation. Which of the following hypotheses are plausible? **(30 points)**
(Select all correct options.)

A. The second band is a result of alternative splicing.
B. The second band is a result of the protein requiring post-translational modification which *E. coli* is unable to carry out.
C. The second band is a result of dimerism of proteins 4 and 5 forming a heterodimer.
D. The second band is a result of all the protein 5 added to the well to be longer than usual.
E. The second band is a result of the protein 5 denaturing.
F. The second band is a result of poor experimental technique introducing hair keratin (a protein) into the sample.

Emma also needs to investigate the interaction between unknown DNA-binding proteins A, B, C, and a DNA fragment. She is also trying to investigate the interactions of X, Y, and Z, which are respectively an antibody that binds to C, a DNA fragment that binds strongly to A, B, and C, as well as a mutated DNA fragment. She adds one of X, Y or Z to lanes 8 to 10, in addition to A, B, C and the DNA fragment. None of these are radioactively labelled.

The DNA fragment was radioactively labelled, mixed with various combinations of proteins as seen in Figure 3, and then subjected to an EMSA. As the gel electrophoresis takes 1h, she walked out of the lab so that she could drink her milk. Unfortunately, Emma spilt milk on her lab notebook again, covering up what she had added into Lanes 8-10. She insisted that it was not her fault as she was drinking milk outside of the lab, which was permitted. Nevertheless, she knows she has to use her results to figure out what she had added to Lanes 8-10.

Lane 1	DNA Ladder (250bp, 500bp, 750bp, 1000bp, 2000bp, 2500bp, 3000bp, 3500bp, 4500bp, 5000bp)
Lane 2	DNA fragment only
Lane 3	DNA fragment + A
Lane 4	DNA fragment + B
Lane 5	DNA fragment + A + B
Lane 6	DNA fragment + B + C
Lane 7	DNA fragment + A + B + C
Lane 8	DNA fragment + A + B + C +
Lane 9	DNA fragment + A + B + C +
Lane 10	DNA fragment + A + B + C +

Figure 3: DNA fragment and proteins added in the respective lanes for EMSA. Milk was spilt on Lanes 8-10 obscuring their information.

The results of the EMSA are shown in Figure 4.

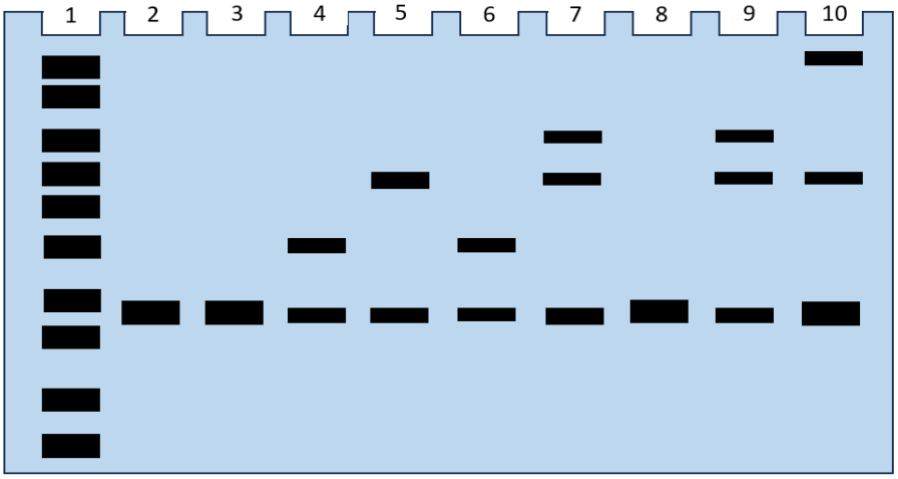


Figure 4: EMSA for unknown DNA-binding proteins

Q3. Indicate whether the following statements regarding the EMSA in Figure 3 are True or False. **(50 points)**

(Mark each statement as true or false.)

A. The DNA fragment is about 3200bp.
B. Just like in SDS-PAGE, the samples were heated with SDS and β-mercaptoethanol to around 100°C before the samples were loaded into the wells for EMSA.
C. The gene that codes for the protein that binds to the DNA fragment in Lane 4 is approximately 1100bp long.
D. The binding efficiency of Protein C is relatively low.
E. If the lane only contained the DNA fragment + A + C, the result would look like Lane 4.

Q4. Help Emma match the letters (A, B, C, X, Y, Z) to the correct questions. **(60 points)**
(Enter the correct letters to the correct rows. If there is more than one letter, input them in alphabetical order or in the order as specified.)

Question	**Letter(s)**
Which protein(s) (A, B, C) can bind to the DNA fragment by itself?	
Which protein (A, B, **or** C) is most likely to be RNA polymerase?	
What is the order of protein binding of A, B and C? *(Indicate the order of binding by typing in the first protein that binds to the DNA fragment, the next protein that binds to this protein, and the last protein that binds to the second one.)*	
Which unknown (X, Y, **or** Z) was added to lane 8?	
Which unknown (X, Y, **or** Z) was added to lane 9?	
Which unknown (X, Y, **or** Z) was added to lane 10?	

Maybe Emma should go for a refresher on safety in the lab with her professor?

Answers and Explanations

Q1.

Answer: **E**

Explanation: Lane 1 is a negative control which shows the band for 100bp. The band is the same for lanes 2, 3 and 4, indicating that the protein did not bind to the DNA fragment, so the DNA fragment is not retarded. The band is shifted back in lane 5, indicating that the DNA-binding domain is from +111 to +137 as its presence allowed the protein to bind to the DNA fragment, thereby retarding its migration, so it appears closer to the wells. The DNA fragment is more retarded in Lane 6 as the protein is larger thus migration is impeded to a larger extent.

Q2.

Answer: **C**

Explanation: Lane 8 contains four bands. The topmost band corresponds to Protein 5 binding, while the third band corresponds to Protein 4 binding. The intermediate band likely corresponds to a Protein intermediate in weight to the two. We want to see why this happens when the two proteins are added together and not when they were added separately.

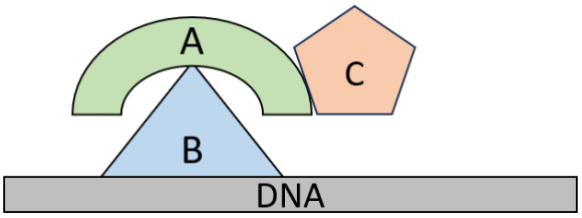
A. If alternative splicing had occurred, the band should also be present in the other lanes.
B. This is irrelevant. Moreover, it should still affect other lanes equally.
C. This is possible. It is likely that both proteins exist as homodimers, so the topmost and third band are likely due to the binding of homodimers 5 and 4 to the DNA respectively. Thus, the intermediate band could be due to the binding of Proteins 4 and 5 together to form a heterodimer, explaining why it was intermediate in weight and approximately exactly in between both bands.
D. There should be no reason for Protein 5 to be longer than usual. Even if it were, it would be heavier, and the additional band should be found closest to the well.
E. If Protein 5 was denatured, it would be unable to bind to the DNA and thus no band by Protein 5 should even be seen.
F. Keratin should not bind to the DNA fragment. Even if it did, keratin is very large and would retard the DNA to a great extent such that the additional band would be the closest to the well.

Analysing the EMSA result

We want to focus on lanes with a retarded band as this would indicate binding. Lane 2 does not have a retarded band, while Lane 3 has a retarded band, implying that Protein A does not bind to the DNA and Protein B binds to the DNA, hence increasing the weight of the protein-DNA complex causing the band to be retarded and appear closer to the well. The band is further retarded in Lane 5 implying that Protein A binds to Protein B which binds to the DNA so the whole protein-DNA complex is even heavier. Lane 6 shows a similar band to that in Lane 4, so Protein C does not bind to the DNA if not there would be one more band or a more retarded band, and Protein C does not bind to Protein B if not the band would be more retarded.

Lane 7 has two bands in addition to the DNA-only band. The intermediate band belongs to Protein A + B binding to the DNA, while the topmost band belongs to all three proteins binding to the DNA. Theoretically, if all C bound to A in the protein A-protein B-DNA complex, there should be no intermediate band as there would be no protein A-protein B-DNA complex left. This implies that C does not have perfect binding efficiency to protein A.

Thus, the binding of the proteins to DNA is seen below:



Now looking at X, Y and Z, X is an antibody which binds to C, so there should be a supershift of the topmost band in Lane 7, which should appear like that in Lane 10. The intermediate band is still there because they do not contain Protein C and are hence not supershifted. Y binds strongly to all three proteins, hence preventing them from binding to the radioactive DNA fragment. Hence there will be no shift and hence there will only be one band like that in Lane 8. Z is mutated and will thus be unable to bind to the proteins and hence will not compete with the radioactive fragment for the proteins. Thus, it will be similar to that in Lane 7, which is Lane 9.

Q3.

Answer: **FFFTF**

Explanation:

A. The DNA ladder is added to Lane 1. The lightest fragment will travel the furthest away from the well. The DNA fragment can be found in between the 750bp and 1000bp DNA ladder fragment, so it is approximately 875bp.
B. β-mercaptoethanol breaks disulfide bonds in proteins. Heating with SDS and β-mercaptoethanol will cause the proteins to denature and they will be unable to bind to the DNA fragments, rendering the whole experiment invalid. Hence, they should not be added.
C. We cannot deduce this from Figure 2. Although the shift corresponds to approximately 1100bp on the gel, this does not imply that the gene coding for the protein is 1100bp long as it is the folded protein that causes the retardation equivalent to 1100bp. The gene of the protein is not tested in the EMSA assay here.
D. As expounded above, theoretically, if all C bound to A in the protein A-protein B-DNA complex, there should be no intermediate band as there would be no protein A-protein B-DNA complex left. This implies that C does not have perfect binding efficiency to protein A. Moreover, the band intensity is similar for both bands, implying that protein C only bound to approximately 50% of all the protein A-protein B-DNA complexes, which is a relatively low binding efficiency.
E. If the lane only contained the DNA fragment + A + C, it will look like lane 3 as neither can bind directly to the DNA fragment, so no shift should be observed.

Q4.

Answer: **B, C, BAC, Y, Z, X**

Explanation: As explained above, only Protein B binds to the DNA fragment. Protein C is most likely RNA polymerase as it binds last in the whole complex. The rest of the questions are explained above.

P33: Despicable Me

(200 points)

Mitogen-activated protein kinases (MAPKs) are a type of protein kinase involved in directing cellular responses to extracellular signals. For example, in mammals, many MAPKs are involved in cell proliferation and mobility coordination. AtMPK10 is a type of MAPK present in *Arabidopsis* plants. To investigate its activity in an *Arabidopsis* plant called "Subject 1", we use a glucuronidase (GUS) reporter. The reporter gene codes for the β-glucuronidase enzyme which can convert non-coloured or non-fluorescent substrates into coloured or fluorescent products, allowing for easy visualisation. Hence, the colour intensity can be used as a surrogate for AtMPK10 activity. The results are seen in Figure 1.

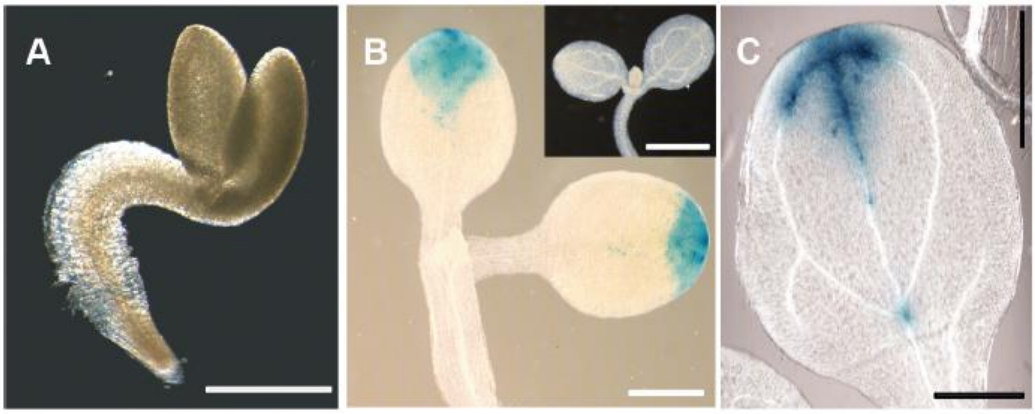


Figure 1: (A) A seedling a day post-germination. (B) A seedling's cotyledons 3 days post-germination (top-right corner: a wild-type seedling with no GUS reporter). (C) One cotyledon for a seedling 12 days post-germination.

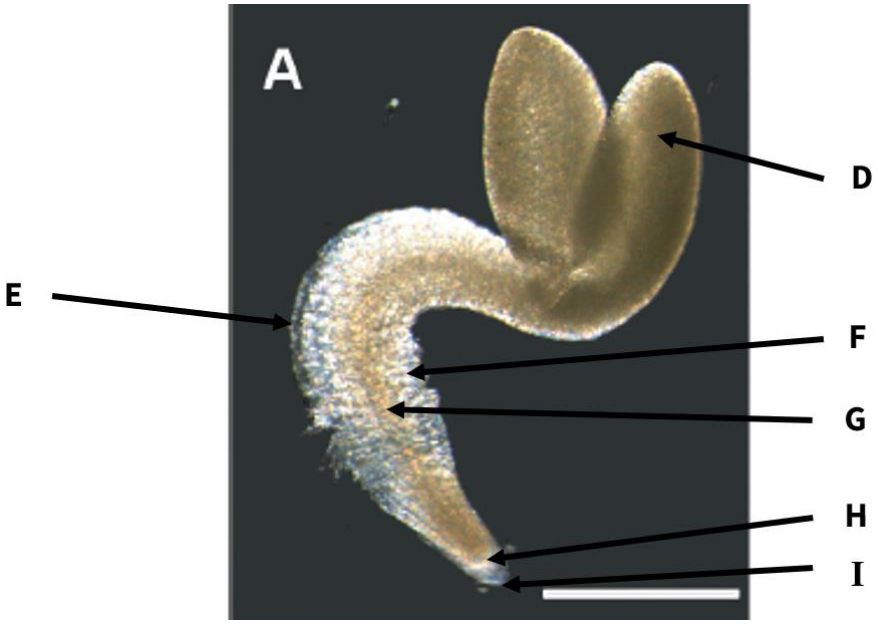


Figure 2: Enlarged labelled diagram of Figure 1A

We then conducted RT-PCR and ran the results on agarose gel with radioactive probes as listed in Figure 3.

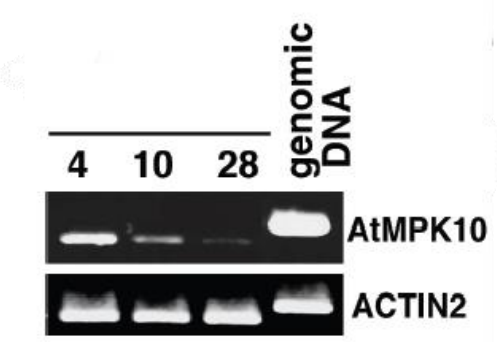


Figure 3: RT-PCR results. Numbers represent the number of days post-germination.

Q1. Match the parts (D-I) in Figure 2 to the correct descriptions below. If there is no such part, enter *None*. Note that not all letters may be used, and some letters may be used more than once. However, there is only one answer to each description. **(40 points)**
(*Match the correct letter to each row.*)

Description	**Part (D-I)**
Protoxylem	
Quiescent centre	
Secretes mucilage	
Endosperm	

Q2. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The cells in I in Figure 2 help determine whether the radicle grows upwards or downwards.
B. AtMPK10 is active 24 hours after a seedling germinates.
C. Active AtMPK10 has an apical distribution.
D. Over time, AtMPK10 activity spreads across the lamina.

Q3. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. AtMPK10 activity slowly becomes restricted to cotyledon veins.
B. As the seedling grows, AtMPK10 expression falls.
C. The venation development in the cotyledons post-germination allows for the sugars made during photosynthesis to be transported from the cotyledons to the rest of the seedling.
D. AtMPK10 tends to be active where leaf veins branch.

After that, we performed a yeast 2-hybrid (Y2H) screening to find out the potential binding partners that AtMPK10 has. The results are seen in Figure 4. Note that AtMKK2 and MKK2 are considered synonymous.

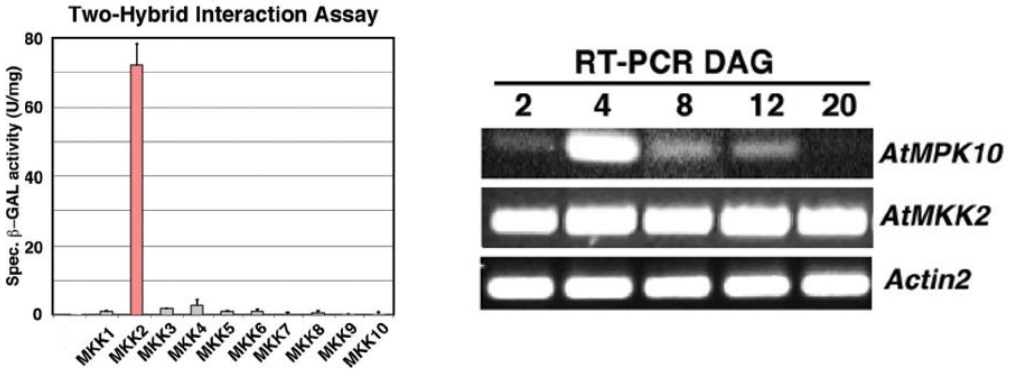


Figure 4: Y2H assay and RT-PCR results. DAG represents days after germination

Q4. Match the following expression levels to the number of days after germination (DAG). **(30 points)**

(*Enter a whole number to each row.*)

Expression level (normalised to highest value)	**DAG**
0.00	
0.15	
1.00	

To investigate some of the MAPK kinases *Arabidopsis* has, we carried out further studies. Figure 5 shows a Y2H assay conducted under different NaCl concentrations with the addition of different MAPKs, while Figure 6 shows a Western blot gauging the activity of MPK4 and MPK6 after the plant is exposed to cold stress.

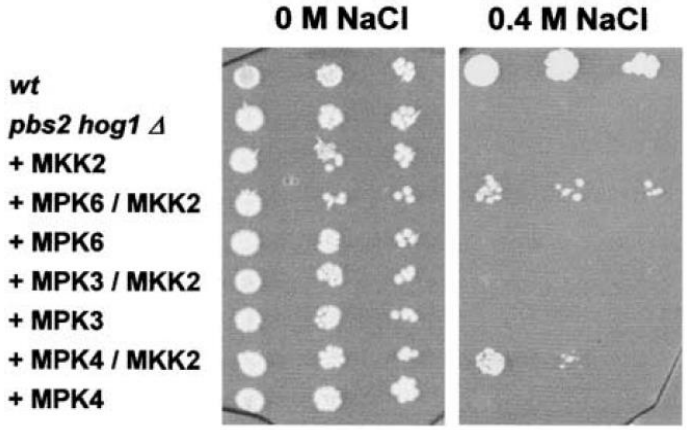


Figure 5: Y2H assay of different MAPKs. *wt* represents wildtype, while *pbs2 hog1 Δ* represents an osmosensitive mutant.

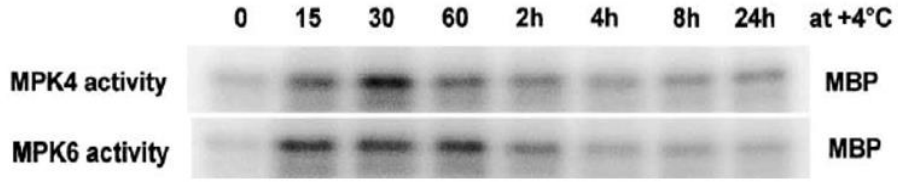


Figure 6: Western blot assay on MAPK4 and MAPK6 activities. MBP as substrate. Intensities are to scale.

Q5. Which of the following MAP kinases interact with MKK2 to allow for salt tolerance? **(10 points)**

(Select all correct options.)

A. MPK3
B. MPK4
C. MPK6

Q6. Indicate whether the following statements are true or false. **(40 points)**

(Mark each statement as true or false.)

A. The assays with added MPK or MKK in Figure 5 were conducted with the *pbs2 hog1 Δ* mutant instead of the wildtype.
B. MPK6 is mobilised before MPK4 to help deal with cold stress.
C. The plant acclimates to cold stress after being in the cold for at least 4h.
D. MPK4 is more active than MPK6 when the plant is exposed to the cold for an hour.

Answers and Explanations

Q1.

Answer: **G, H, I, None**

Explanation:

A. G is the vascular bundle which contains the xylem and physiologically older protoxylem.
B. H is the quiescent centre which do not divide mitotically.
C. I is the root cap which secrete mucilage to protect the delicate root apical meristem.
D. The endosperm is not labelled.

Q2.

Answer: **TFTF**

Explanation:

A. Statoliths in the root cap aid with gravitropism and allows the germinating seedling to sense upwards and downwards, thus allowing the radicle to grow downwards to the soil.
B. No blue spots are observed in the seedling yet.
C. In figures 1B and 1C, we see the blue spots growing from the apex.
D. From figures 1B and 1C, it becomes restricted to the leaf veins.

Q3.

Answer: **TTFT**

Explanation:

A. This is clearly seen from figure 1B to figure 1C.
B. Figure 3 shows the band intensity decreasing over time.
C. Cotyledons do not undergo photosynthesis.
D. This can be seen from figure 1C as activity is higher at branch points.

Q4.

Answer: **20, 2, 4**

Explanation:

A. 0.00 – No visible band is seen on day 20.
B. 0.15 – We can approximate this based on the other band intensities.
C. 1.00 – The most intense band is seen on day 4.

Q5.

Answer: **B, C**

Explanation: Since only yeast with those genes could survive and grow on the high salt concentration media, these genes allow for salt tolerance.

Q6.

Answer: **TTTF**

Explanation:

A. If it were conducted with the wildtype, we would see colonies for all experiments as the wildtype is able to tolerate salt. It would only make sense to use the mutant which is not salt tolerant to see specifically which genes can confer salt tolerance to the yeast mutant cells.
B. MPK6 becomes active after 15min while MPK4 becomes active after 30min. We can see this by observing the band intensity levels in Figure 6.
C. After 4 hours of cold exposure, MPK4 and MPK6 activities return back to basal levels, suggesting acclimatisation.
D. At the 60min mark, the band intensity for MPK4 is lower than that of MPK6 and is hence less active.

Credits

Figures 1, 2, 3, 4: Modified from Stanko, V., Giuliani, C., Retzer, K., Djamei, A., Wahl, V., Wurzinger, B., Wilson, C., Heberle-Bors, E., Teige, M., & Kragler, F. (2014). Timing is everything: highly specific and transient expression of a MAP kinase determines auxin-induced leaf venation patterns in Arabidopsis. *Molecular plant*, *7*(11), 1637–1652. https://doi.org/10.1093/mp/ssu080

Figures 5, 6: Modified from Teige, M., Scheikl, E., Eulgem, T., Dóczi, R., Ichimura, K., Shinozaki, K., Dangl, J. L., & Hirt, H. (2004). The MKK2 Pathway Mediates Cold and Salt Stress Signaling in Arabidopsis. *Molecular Cell*, *15*(1), 141–152. https://doi.org/10.1016/j.molcel.2004.06.023

Content reference: Cargnello, M., & Roux, P. P. (2011). Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiology and molecular biology reviews : MMBR*, 75(1), 50–83. https://doi.org/10.1128/MMBR.00031-10

P34: Character Displacement

(120 points)

Pogoniulus bilineatus and *Pogoniulus subsulphureus* are 2 closely related species. *Pogoniulus bilineatus* are typically found in more open habitats and *Pogoniulus subsulphureus* are found in dense forests. Their geographical range tended not to have overlapped in the past (allopatry) but may have started to coexist in areas (sympatry) where pristine rainforest has been degraded due to deforestation. This had led to morphological and behavioural changes in both species. Bird songs are typically sung by male birds to attract mates or signal territorial ownership to their conspecifics.

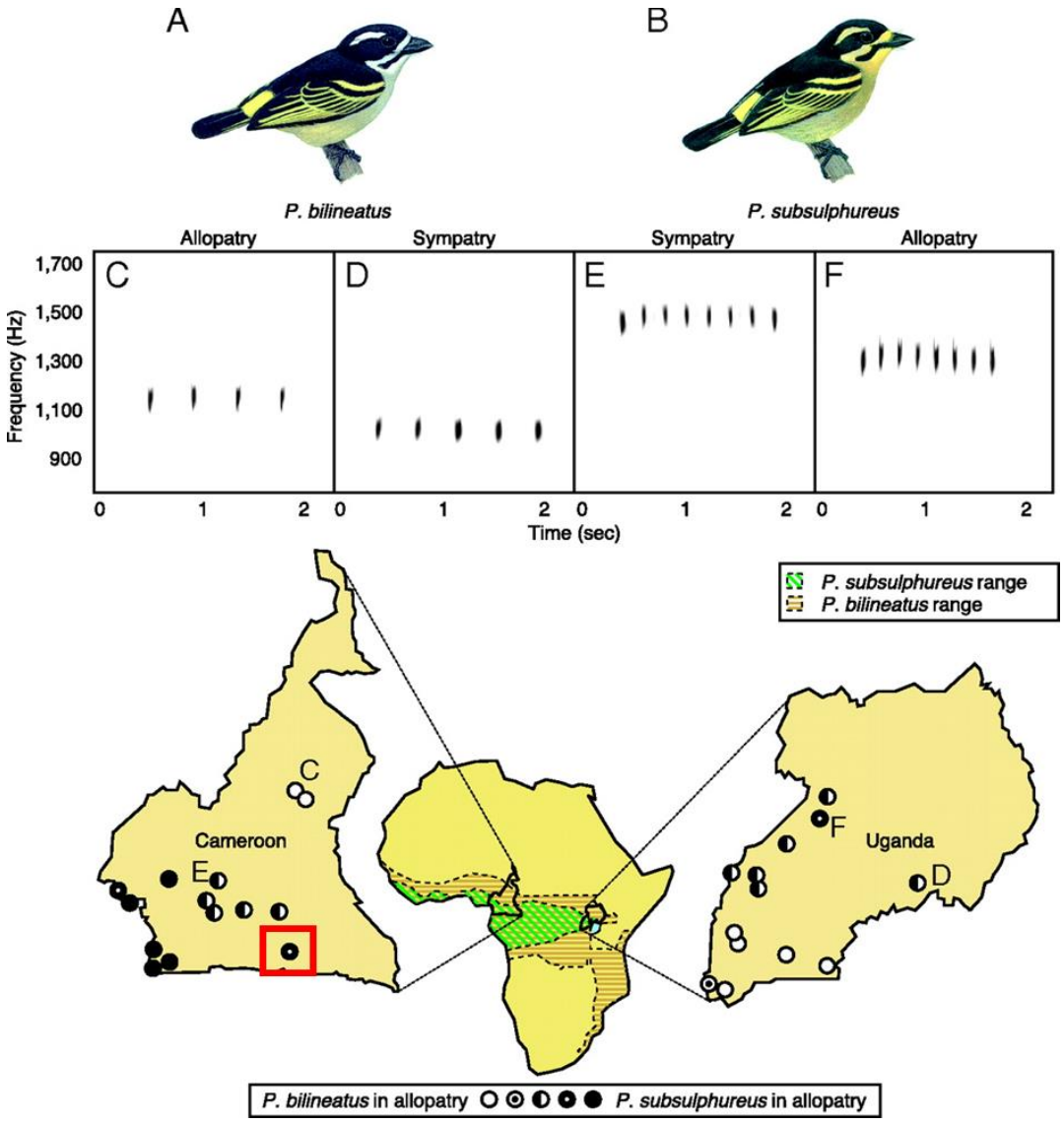


Figure 1: (C–F) Spectrograms of bird songs. The Africa map illustrates the species' distributions. Each circle represents a habitat where either one or both species are present. Site locations are illustrated for *P. bilineatus* in allopatry (white circles); *P. subsulphureus* in allopatry (black circles); *P. subsulphureus* common, *P. bilineatus* rare in sympatry (black circles with white dots); *P. bilineatus* common, *P. subsulphureus* rare in sympatry; and both species common in sympatry (half-filled circles).

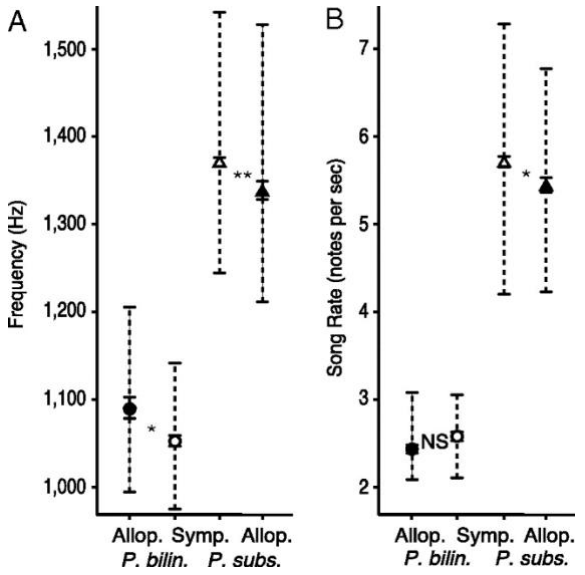


Figure 2: Frequency and song rate of *P. subsulphureus* and *P. bilineatus* in sympatry and allopatry.

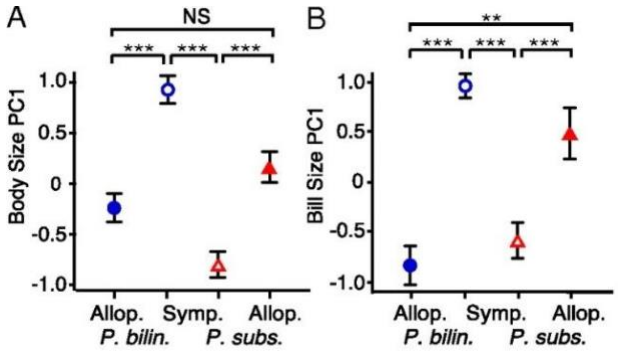


Figure 3: Body size and bill size of *P. subsulphureus* and *P. bilineatus* in sympatry and allopatry.

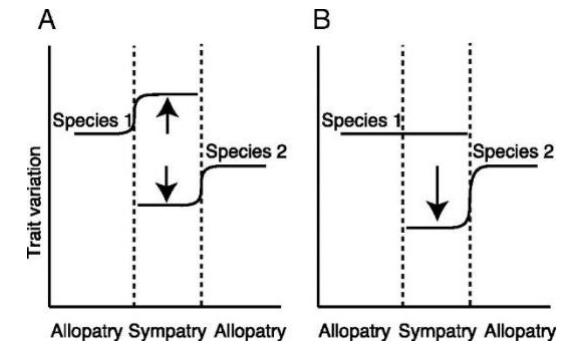


Figure 4: Effect of evolutionary-related, ecologically-similar heterospecifics on evolution of traits of its counterpart when in sympatry may vary based on relative abundance.

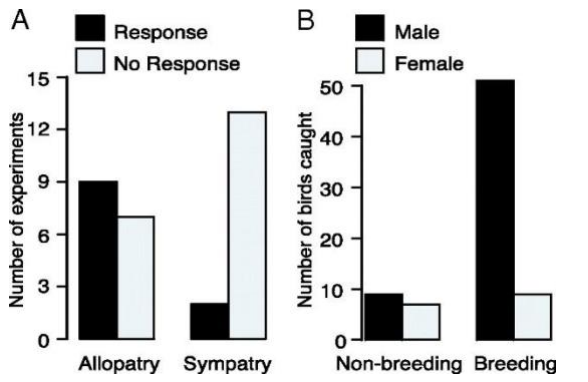


Figure 5:
A: Synthetic mean heterospecific songs were played and the number of responses were measured.
B: Synthetic mean conspecific songs were played and the number of birds caught were measured.

Q1. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. In both Cameroon and Uganda, there are more habitats with birds in sympatry than allopatry.
B. Both species had a change in song rate from its allopatric condition when in sympatry.
C. Frequency of notes is likely a more significant differentiator in song types compared to song rate.
D. The data supports the acoustic adaptation hypothesis.

Q2. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. Frequency of songs is generally positively correlated with song rate.
B. An increase in body and bill size is correlated with a lower pitch when comparing different populations of the same species.
C. Deforestation is leading to phenotypic convergence between *Pogoniulus bilineatus* and *Pogoniulus subsulphureus*.
D. *Pogoniulus bilineatus* and *Pogoniulus subsulphureus* in allopatry had significantly different body size and bill size.

Q3. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The reproductive barrier between *Pogoniulus bilineatus* and *Pogoniulus subsulphureus* is stronger in allopatry than in sympatry.
B. *Pogoniulus subsulphureus* in the habitat which is boxed in red in Figure 1 is species 2 in Figure 4B for the area of sympatry.
C. Vocalisation of bird songs where the 2 birds are in sympatry are used to mark territorial boundaries against heterospecifics (e.g. *Pogoniulus bilineatus* warding off *Pogoniulus subsulphureus*).
D. The males are singing during the breeding season primarily to attract mates.

Answers and Explanations

Q1.

Answer: **FFTF**

Explanation:

A. In Cameroon, 7 habitats in allopatry, 7 habitats in sympatry. In Uganda, 5 habitats in allopatry, 8 habitats in sympatry. Hence, the statement is false for Cameroon.
B. The change was not significant for *Pogoniulus bilineatus*.
C. There was a more significant change in song frequency than song rate.
D. The acoustic adaptation hypothesis predicts that lower frequencies are favored in dense forests because they propagate better through the cluttered environment of thick vegetation. Lower frequency sounds experience less attenuation and distortion caused by foliage, branches, and trunks, allowing the sound to travel farther and remain clearer for communication purposes. However, the bird songs of *Pogoniulus subsulphureus*, which lives in dense forests, are found to have a higher frequency.

Q2.

Answer: **TTFF**

Explanation:

A. *Pogoniulus subsulphureus* had both a higher frequency and song rate than *Pogoniulus bilineatus*.
B. *Pogoniulus subsulphureus* in sympatry had both a lower song rate and frequency than its counterparts in allopatry. *Pogoniulus bilineatus* in sympatry had both a higher song rate and frequency than its counterparts in allopatry.
C. Deforestation is leading to sympatry, which has been driving phenotypic divergence, as *Pogoniulus subsulphureus* and *Pogoniulus bilineatus* in sympatry had greater phenotypic differences than *Pogoniulus subsulphureus* and *Pogoniulus bilineatus* in allopatry.
D. Difference in body size between *Pogoniulus subsulphureus* and *Pogoniulus bilineatus* in allopatry is not significant.

Q3.

Answer: **FFFF**

Explanation:

A. Generally, the reproductive barrier between 2 closely related species is stronger in sympatry than allopatry. In this case, it can be supported by the fact that there is greater phenotypic divergence between *Pogoniulus subsulphureus* and *Pogoniulus bilineatus* in sympatry than in allopatry.
B. Species 2 in Figure 4B underwent a change in its phenotype, while species 1 did not. This suggests that species 1 is more abundant and hence is relatively unaffected by the presence of species 2, while species 2 is less abundant. For the habitat boxed in red, species 2 is *Pogoniulus bilineatus*, not *Pogoniulus subsulphureus*.
C. As seen from Figure 5A, birds generally do not respond to bird songs of heterospecifics.
D. Males responded more than females, which suggests that bird songs are predominately used as a means to mark territory, rather than to attract mates.

Credits

Figures 1, 2, 3, 4, 5: Kirschel, A. N., Blumstein, D. T., & Smith, T. B. (2009). Character displacement of song and morphology in African Tinkerbirds. *Proceedings of the National Academy of Sciences*, *106*(20), 8256–8261. https://doi.org/10.1073/pnas.0810124106

P35: Replication: A Closer Look

(190 points)

Overview of DNA Replication

DNA replication occurs semi-conservatively, with each parental strand serving as a template for the synthesis of a new daughter strand. As DNA polymerase can only synthesise DNA in the 5'-to-3' direction, this creates Okazaki fragments, which are short segments of nucleotides formed during DNA replication (Figure 1).

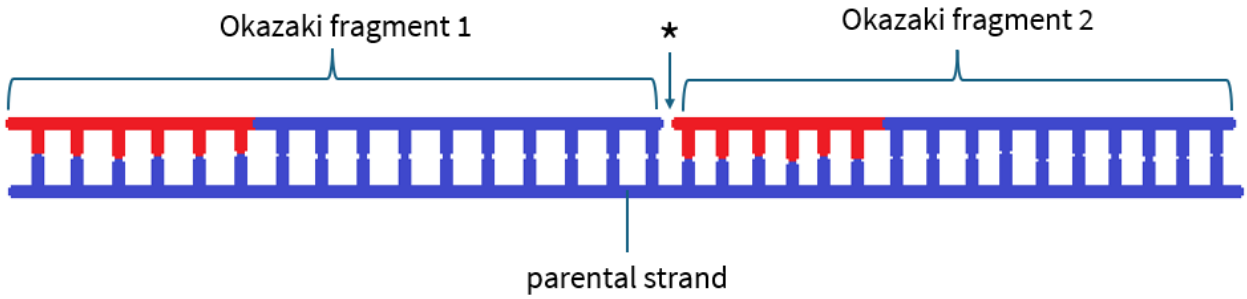


Figure 1: DNA replication. Blue: DNA, Red: RNA

Q1. Which statement about DNA replication is true? **(20 points)**
(Select the correct option.)

A. In *E. coli*, the Okazaki fragments on the lagging strand are synthesised in the same direction in which the replication fork is progressing.
B. The synthesis of the lagging strand "lags" behind that of the leading strand as the DNA polymerase that synthesises the lagging strand has a slower rate of synthesis (i.e. fewer nucleotides incorporated per second).
C. After the removal of RNA primers but prior to the action of DNA ligase, there will be a gap in the sugar-phosphate backbone at the position marked with an asterisk (*) between Okazaki fragments 1 and 2 in Figure 1.
D. When fully synthesised, a single daughter DNA strand comprises parts that were synthesised continuously and parts that were synthesised discontinuously during DNA replication.

Back to Where It All Started: Finding the Origin

DNA replication begins at specific DNA sequences called origins of replication. Two-dimensional DNA electrophoresis can be used to determine the position of the origin of replication. After restriction digest, replicating DNA molecules are separated by size in the first dimension, and then by shape in the second dimension. Results of one such experiment are shown in Figure 3.

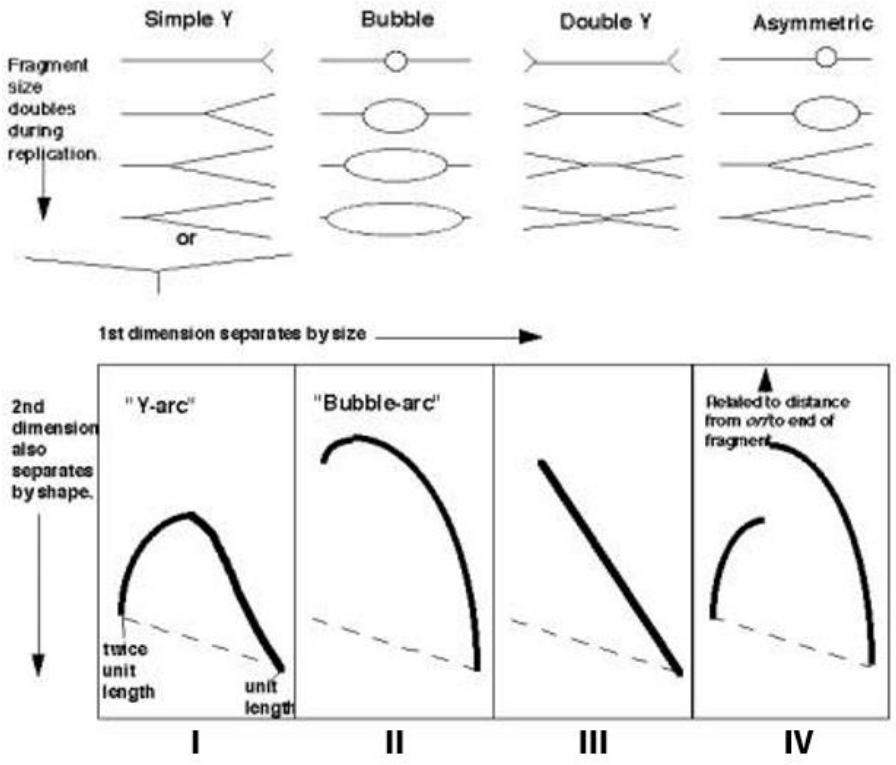


Figure 2: Principles of 2-dimensional DNA electrophoresis. The corresponding gel (I, II, III, IV) is shown below each type of DNA fragment.

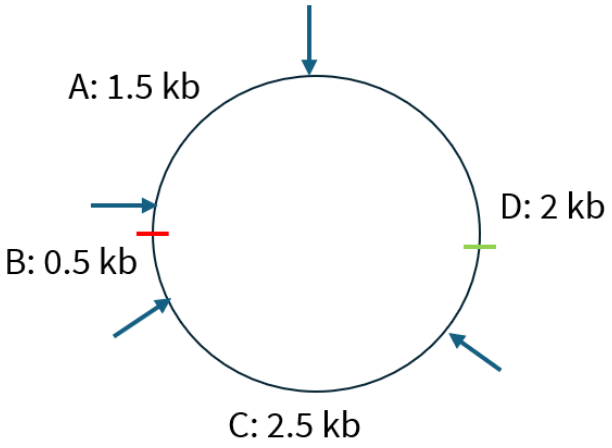


Figure 3: Restriction map of plasmid containing a single origin of replication and a single terminator sequence. Arrows: Restriction sites, Green hatch mark: Origin of replication, Red hatch mark: Terminator sequence

Q2. Match the fragments (A, B, C, D) in Figure 3 with their corresponding gel results (I, II, III, IV) in Figure 2. Note that each Roman numeral may be used more than once or not at all. **(40 points)**
(Match the correct Roman numerals to the correct letters.)

Fragment	Gel Result
A	
B	
C	
D	

Cell Replication

The cell cycle, the process by which a cell replicates its DNA and divides into two genetically identical daughter cells, consists of the G_1, S, G_2 and M phases. Three experiments were conducted to determine the length of each phase of the cell cycle for a culture of a hypothetical microbe. In experiment 1, the number of viable cells in a growth medium were counted at 20-hour intervals (Figure 4). In experiment 2, the cells were first briefly exposed to radioactive thymidine, washed, and re-incubated with non-radioactive thymidine. The percentage of mitotic cells that were labelled were counted at one-hour intervals (Figure 5). In experiment 3, microscopic observation showed 10% of cells to be in M phase.

Time/h	log(number of cells)
0	1
20	1.2

Figure 4: Experiment 1 Results.

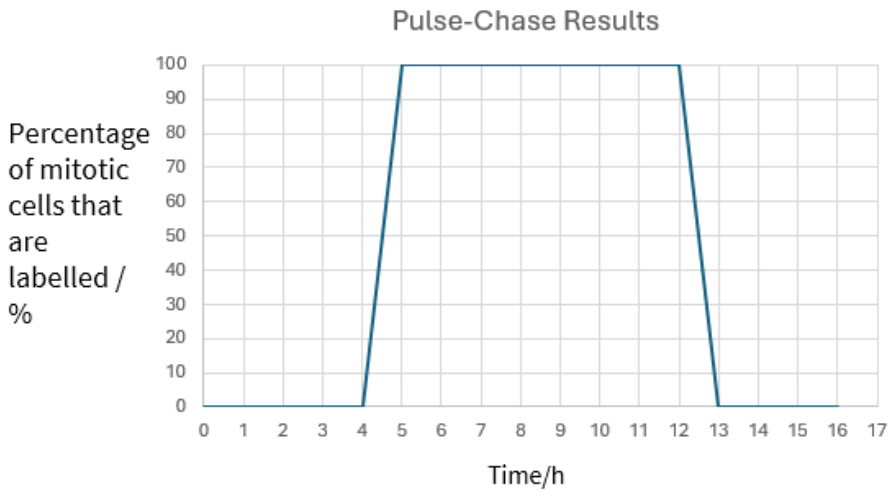


Figure 5: Experiment 2. Time = 0 h indicates the time when radioactive thymidine was added.

Q3. To the nearest whole number, what is the total duration of the entire cell cycle? **(20 points)**
(Select the correct option.)

A. 9 h
B. 18 h
C. 26 h
D. 30 h

Q4. Calculate the duration of the M phase in hours. Round off your answer to the nearest whole number. **(10 points)**
(Enter your answer correct to the nearest whole number. Do not include any units.)

Q5. Calculate the duration of the G_1, S and G_2 phases in hours. Round off all answers to the nearest whole number. **(60 points)**
(Enter your answer correct to the nearest whole number to each row.)

Phase of Cell Cycle	**Duration in hours**
G_1	
S	
G_2	

E. coli Replication

Replication of the entire *E. coli* genome takes 40 min to complete. Under normal circumstances, regulatory mechanisms ensure that the origin of replication is only initiated once during each round of cell division, coupling the process of DNA and cell replication. In *E. coli*, one such mechanism is the methylation of the DNA sequences at the origin of replication. Immediately after DNA replication, at the origins of replication, the daughter strands are unmethylated, whereas the parental strands are methylated. The next round of DNA replication can only be re-initiated after the daughter strands are methylated, which only occurs a while after DNA replication is complete.

A student cultured *E. coli* in a particular growth medium, and determined the generation time to be 20 min. The student observed the replicating *E. coli* genome under the microscope and drew the structure shown in Figure 6.

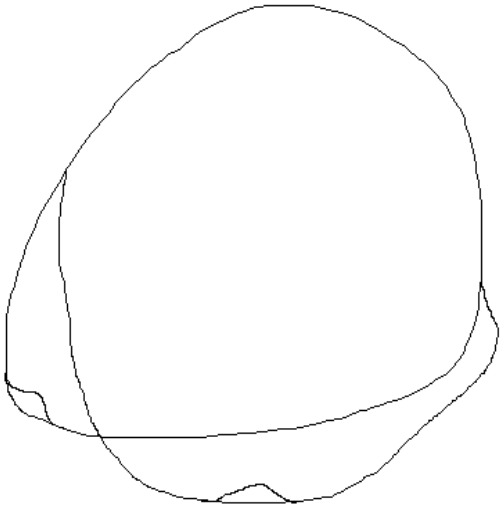


Figure 6: *E.* coli genome.

Q6. Indicate whether the following statements regarding replication of the *E. coli* genome are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The students' observations in Figure 6 are due to DNA supercoiling (the twisting of the DNA double helix onto itself).
B. A mutation in topoisomerase can explain the results, as it prevents concatenated DNA (interlocked DNA circles) from being resolved.
C. The results indicate the presence of chromosome dimers.
D. It is likely that the inhibition of DNA replication initiation is overcome in *E. coli* cells growing in this medium.

Answers and Explanations

Q1.

Answer: **D**

Explanation:

A. The lagging strand is synthesised as a series of Okazaki fragments in the direction opposite to the replication fork.
B. The reason why the synthesis of the lagging strand “lags” is that sufficient nucleotides on the template strand must first be exposed before the synthesis of a new Okazaki fragment can begin.
C. The gap will now be at the original junction between the RNA primer and Okazaki fragment 2, as the RNA primer will be removed in the 5’-to-3’ direction and new dNTPs added to the 3’ end of Okazaki fragment 1.
D. If on one replication fork within the replication bubble the daughter DNA strand is synthesised as a leading strand, then it will be synthesised as a lagging strand on the other replication fork within the same replication bubble, and vice versa.

Q2.

Answer: **I, III, I, IV**

Explanation:

A. There is only one origin of replication (that is within fragment D) and terminator sequence (that is within fragment B), so A contains neither terminator sequence nor the origin of replication. Hence, A produces the Y arc pattern characteristic of a single replication fork at one end.
B. B contains the terminator sequence with one replication fork at each end.
C. Same reasoning as A
D. Origin of replication is not located in the middle of fragment D.

Q3.

Answer: **D**

Explanation:

$$10^{1.2} = 10 \times 2^n \ (exponential\ population\ growth\ equation)$$

$$1.2 = 1 + n\ lg\ 2 \ \Rightarrow \ 0.2 = n\ lg\ 2$$

$$n = \frac{0.2}{\lg 2} \ (number\ of\ generations)$$

$$t = \frac{20h}{n} = 30h \ (total\ length\ of\ cell\ cycle)$$

Q4.

Answer: **3**

Explanation:

$$M\ phase \ = \ 10\% \times 30h \ = 3h$$

Q5.

Answer: **14, 9, 4**

Explanation:

Note that the cells are not synchronised, that is, they may be at different stages of the cell cycle when radioactive thymidine was added. Thus, only cells that were in S phase and hence replicating their DNA when the radioactive thymidine was added would incorporate it into their DNA and become labelled.

The percentage of mitotic cells that were labelled first begins to rise at time = 4h. The cells responsible for this initial rise are those that were about to complete S phase when radioactive thymidine was added. The time between S phase and M phase corresponds to the G2 phase, which thus lasts 4h.

Similarly, the percentage of mitotic cells labelled falls to zero at time = 13h. These cells are those that just entered S phase when radioactive thymidine was added. The time between the initial rise and the decrease to zero thus corresponds to the duration of S phase, which is 9h.

$$G1\ phase\ =\ 30-4-9-3=14h$$

Q6.

Answer: **FFFT**

Explanation:

The so-called replication paradox lies in how the time needed to replicate the entire bacterial genome is longer than the time required for cell division. The underlying basis for this phenomenon is that under certain conditions, *E. coli* may re-initiate DNA replication at the origin of replication before the previous round of DNA replication is complete, producing nested replication forks as seen in Figure 6. Thus, statement D is the only true statement, as the re-initiation of replication necessitates overcoming the normally inhibitory mechanism of methylation. Statement B is false as that would show the two sets of chromosomes being interlocked.

Credits

Figure 2: *Structural analysis of pulse-labeled DNA molecules*. CHAPTER 6. (n.d.). https://www.bx.psu.edu/~ross/workmg/Replication2Ch6.htm

P36: T6

(210 points)

Construction of Changi Airport's T5 is starting in 2025. I wonder when is T6 going to be built?

In mammalian X-chromosome inactivation (XCI), one of two X chromosomes is randomly inactivated early during embryonic development as a mechanism for dosage compensation of sex chromosome genes. One X chromosome becomes inactivated to form the Barr body, and only genes on the other X chromosome are expressed. This XCI process is regulated by the Xist (X-inactive specific transcript) RNA. Once XCI has occurred, this epigenetic pattern is faithfully inherited by all progeny cells.

Calico cats have both orange and black patches, which occurs due to XCI. Hence, some patches will be orange due to expression of the allele which codes for orange fur, while the other patches will be black due to the other allele which codes for black fur.

Figure 1: Calico Cat

Q1. You are surprised to find that the calico cat you have has a penis. Which of the following statements are plausible explanations for the observed phenotype? Indicate True if the explanation is plausible and False if it is not plausible. **(50 points)**
(Mark each statement as true or false.)

A. The genotype of the cat is XXY.
B. Non-disjunction of sex chromosomes in anaphase II occurred in the germ cell that gave rise to the egg which formed the cat.
C. While the cat has XY genotype, unequal crossing-over occurred in oogenesis in the mother, which resulted in two genes that code for fur colour (one coding for orange pigment and one coding for black pigment) being found on the X chromosome.
D. The cat has XX genotype but also has congenital adrenal hyperplasia, so the adrenal gland produces high levels of testosterone.
E. The cat has XY genotype but the Y chromosome happens to carry the gene that codes for fur colour.

XCI is a form of sex chromosome gene dosage compensation to balance the levels of X-linked gene products between both sexes. The mechanism of dosage compensation can differ greatly between species. In marsupial mammals like koalas, the paternally-derived X chromosome is specifically inactivated, while in the fruit fly *Drosophila melanogaster*, the level of gene expression on X chromosomes in males is doubled. Meanwhile, in the roundworm *Caenorhabditis elegans*, the level of gene expression on X chromosomes on hermaphrodites is halved.

The difference in mechanisms can be determined by carrying out sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the glucose-6-phosphate dehydrogenase (G6PD) enzyme. The G6PD enzyme is encoded by a single gene found on the X chromosome. This gene has two alleles, one A_1 allele, which codes for a G6PD isozyme, and a mutant A_2 allele, which codes for a different isozyme, due to 102 fewer nucleotides compared to the A_1 allele.

You decided to conduct a test on six different organisms to distinguish their dosage compensation mechanisms. Each organism had the A_1 allele on one X chromosome and the A_2 allele on the other X chromosome. You randomly extracted four cells from each organism and homogenised each cell separately. The G6PD enzymes of each cell sample were extracted and purified, and then subjected to SDS-PAGE. Assume all organisms are heterozygous for the G6PD gene where possible.

Figures 2 to 5 show the different possible SDS-PAGE results.

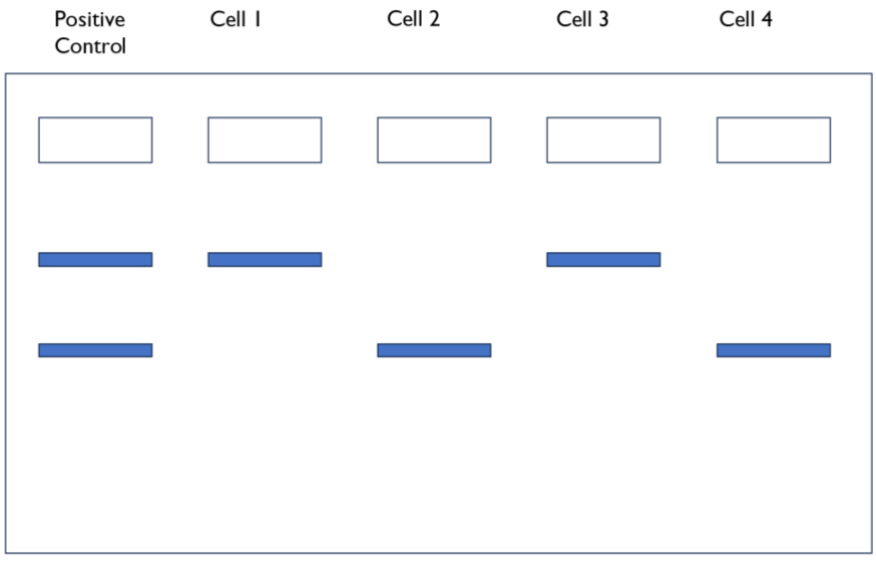


Figure 2

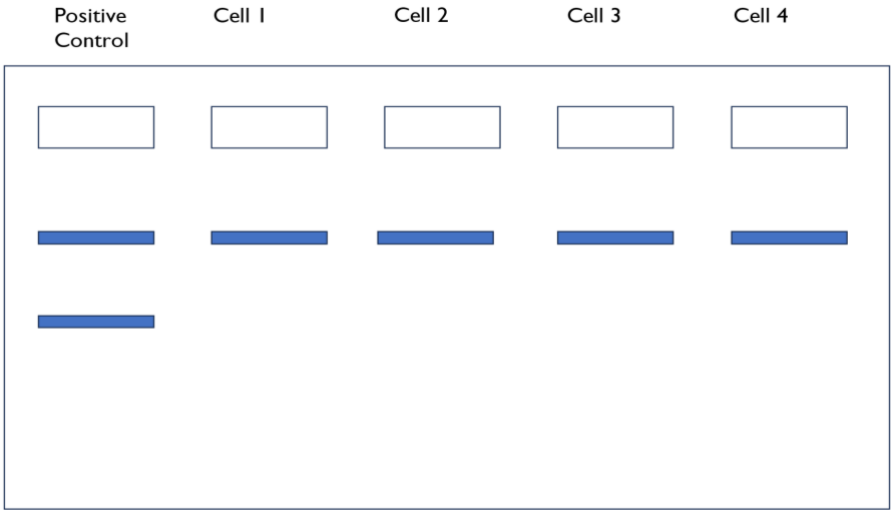


Figure 3

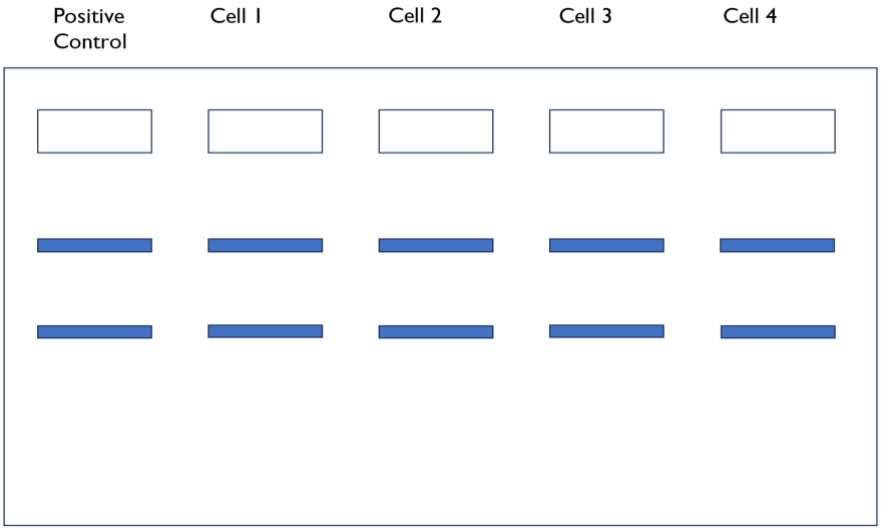


Figure 4

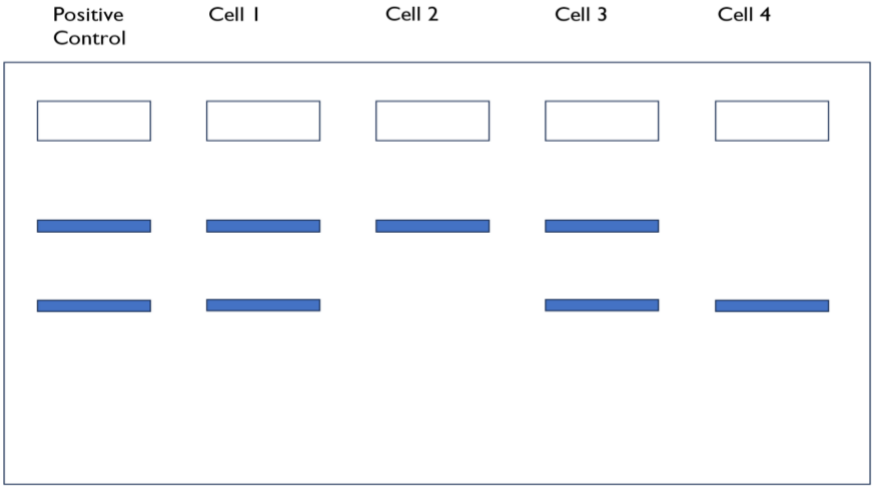


Figure 5

Q2. Indicate the expected SDS-PAGE results for the following organisms by indicating the correct figure numbers (2-5). Figure numbers may be used once, more than once or not at all. **(50 points)**
(Match the correct number to the correct row.)

Organism	**Expected Result (2-5)**
Normal human female	
Human female with Turner syndrome	
Caenorhabditis elegans hermaphrodite	
Marsupial female	
Drosophila melanogaster female	

G6PD dimers are produced in cells. First, transcription and translation occur to produce the G6PD monomers. Two G6PD monomers dimerise to form a G6PD dimer, which is then secreted into the blood. Sometimes under certain conditions, the dimers can also dimerise forming tetramers.

Q3. If a woman has both alleles A_1 and A_2 for G6PD, what is the proportion of A_1A_1 dimers found in her blood? **(20 points)**
(Enter your answer as a decimal correct to 3 s.f.)

XCI can occur for either the paternal or maternal chromosome. Which chromosome is inactivated is dependent on the X-inactive specific transcript (Xist) gene which encodes a lncRNA (long non-coding RNA). The X-chromosome that expresses the Xist gene is coated by the lncRNA, allowing it to be inactivated.

Q4. The inactivated X-chromosome is isolated from a cell. What is the easiest way for you to determine whether this inactivated X-chromosome is paternal or maternal? **(20 points)**

A. Karyotype with staining
B. Co-immunoprecipitation
C. Restriction fragment length polymorphism (RFLP)
D. Microarrays
E. DNase-seq
F. Sanger sequencing
G. Yeast-2-hybrid
H. Southern Blot

Q5. In general, the probability of XCI of either parental chromosome is 50%. If random XCI occurs at the 8-cell stage, calculate the probability that there are an equal number of cells expressing each X chromosome. **(20 points)**

(Enter your answer as a decimal correct to 3 s.f.)

Q6. Now assume that random XCI occurs at the 64-cell stage. Calculate the probability that there are between 30 and 34 cells (inclusive) with the paternal X chromosome expressed at the 64-cell stage. (Hint: You may find the use of a graphing calculator helpful.) **(30 points)**

(Enter your answer as a decimal correct to 3 s.f.)

Your professor posed a question regarding XCI at the 64-cell stage to the class for discussion:

At the 64-cell stage, is the proportion of cells with the paternal X chromosome expressed **likely to be closer to**, **equal to**, or **further from 50%** than that in the 8-cell stage?

Dr Jerome (The professor)

It's clearly closer to 50%. Since 8 is not a large sample size, the binomial distribution of proportion of cells with paternal X-chromosome expressed is not approximately normal by the Central Limit Theorem, so the proportion of cells with paternal X chromosome expressed is more likely to deviate from the mean for the 8-cell stage.

Owen

Alicia

Okay, I have no idea what Dr Jerome is saying. This is not possible to predict because X-chromosome inactivation is a random event so the likelihood is random. It's like saying if it is more likely for Owen or me to find an apple to feed on today. An apple a day keeps the doctor away; hope I can find one to keep Dr Jerome from asking such odd questions in the future.

I disagree with Owen and Alicia. The answer should be "equal to 50%". Since whether a cell has a paternal or maternal X chromosome expressed is a random, independent event, the mean and standard deviation of the normal distribution representing the proportion of cells with the paternal X chromosome expressed remains the same regardless of the stage where X-chromosome inactivation occurs.

Cervon

Hey that's not fair! Cervon has a laptop to search for the answer! He may have gotten the correct answer, but his explanation is wrong! As the number of random, independent events of X-chromosome inactivation occurring increases, the observed number of cells with paternal X-chromosome expressed will not converge towards the expected number of cells with paternal X-chromosome expressed according to the Law of Large Numbers.

Yuting

Hey! Don't blame him; we can use our laptops just like during the SBL! However, the answer is actually "further from 50%". We literally learnt this before in class! As the number of random, independent events (whether a cell has a paternal or maternal X chromosome expressed) increases, variance increases leading to greater likelihood of deviation from the mean.

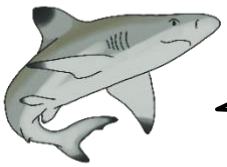
Le Xuan

You all yap so much but the answer is trivial: Closer to 50%. This is because of the Law of Large Numbers. As the number of random, independent events of X-chromosome inactivation occurring increases, the proportion of cells with the paternal X chromosome expressed converges to the mean.

Debraath

Actually, the answer is "further from 50%". Let me explain. Since 8 is not a large sample size, the binomial distribution of proportion of cells with paternal X-chromosome expressed is not approximately normal by the Central Limit Theorem, so the proportion of cells with paternal X chromosome expressed is less likely to deviate from the mean for the 8-cell stage.

Ethan



HAHA this is a trick question! X-chromosomal inactivation has been recently proven by N. Sens et al. (2023) to only occur in some mutants. X-chromosomal inactivation rarely occurs so this question is actually invalid!

Lionel

That's a great discussion everyone! However, only one of you had the correct answer and explanation to my question. Come find me after class to get a chocolate bar as your prize!

Dr Jerome (The professor)

Q7. Which student had both the correct answer and explanation to Dr Jerome's question and will hence be receiving a chocolate bar from him? **(20 points)**

(Select the correct option.)

A. Owen
B. Alicia
C. Cervon
D. Yuting
E. Le Xuan
F. Debraath
G. Ethan
H. Lionel
I. Dr Jerome is disappointed and everyone was wrong

Answers and Explanations

Q1.

Answer: **TFFTF**

Explanation:

A. Presence of Y chromosome results in a male cat with a penis. Random X chromosome inactivation occurs due to 2 X chromosomes.
B. Nondisjunction in anaphase II will result in both X chromosomes having the same allele for fur colour, leading to a uniform coat.
C. No X chromosome inactivation occurs as there is only 1 X chromosome. This leads to both alleles being expressed and all cells in the epidermis will be of the same colour, leading to a uniform coat (although the fur colour may not be strictly black or orange but something in between).
D. Congenital adrenal hyperplasia leads to synthesis of testosterone by the adrenal cortex, which results in virilisation and male reproductive organs forming for the female cat. Random X inactivation occurs which leads to the calico phenotype.
E. Phenotype will be the same as in the case of C as no random X chromosome inactivation occurs.

Q2.

Answer: **23434**

Explanation:

A. A normal female will have random X inactivation, so only 1 of the 2 alleles will be expressed in each cell and only 1 of the 2 isozymes is found.
B. A female human with Turner syndrome has genotype XO, so the only X chromosome will be expressed, resulting in all cells having the same allele and hence the same isozyme is found in all the cells.
C. In the roundworm *Caenorhabditis elegans*, the level of gene expression on X chromosomes on hermaphrodites is halved, meaning that both alleles are expressed and both isozymes are found in all cells.
D. In marsupial mammals like koalas, the paternally-derived X chromosome is specifically inactivated, resulting in all cells expressing the maternal allele and hence the same isozyme is found in all the cells.

E. In the fruit fly *Drosophila melanogaster*, the level of gene expression on X chromosomes in males is doubled, so in females, both X chromosomes have normal expression, so both alleles are expressed and both isozymes are found.

Q3.

Answer: **0.500**

Explanation: Since two G6PD monomers dimerise to form a G6PD dimer *within cells*, it means A_1A_2 heterodimers are *not possible*, as only exactly one of A_1 or A_2 allele is expressed in each cell. Hence, 50% of dimers will be A_1A_1 and 50% will be A_2A_2.

Q4.

Answer: **C**

Explanation: Only C, F and H work to differentiate paternal and maternal chromosomes. Among the 3 molecular techniques, RFLP is the simplest.

Q5.

Answer: **0.273**

Explanation: We need to get 4 cells expressing paternal X chromosome (probability is $\left(\frac{1}{2}\right)^4$) and 4 cells expressing maternal X chromosome (probability is $\left(\frac{1}{2}\right)^4$). However, they are unordered cells, so we multiply the probability by ${}^8C_4$.

$$Probability = \binom{8}{4} * \left(\frac{1}{2}\right)^8 = 0.2734375$$

Q6.

Answer: **0.468**

Explanation: Using the same logic as above, we need to get n cells expressing paternal X chromosome (probability is $\left(\frac{1}{2}\right)^n$, where n is between 30 and 34 and $64-n$ cells expressing maternal X chromosome (probability is $\left(\frac{1}{2}\right)^{64-n}$). However, they are unordered cells, so we multiply the probability by ${}^{64}C_n$ for each n.

$$Probability = binomcdf(64, 0.5, 34) - binomcdf(64, 0.5, 30) = 0.468$$

OR

$$Probability = \sum_{30}^{n=34} \binom{64}{n} \left(\frac{1}{2}\right)^{64} = 0.468$$

Q7.

Answer: **F**

Explanation: Deviation of proportion of cells with the paternal X chromosome decreases from the average of 0.5 as n increases, according to the Law of Large Numbers.

The derivation is as follows for the mathematically-inclined.

Let X be the number of cells with paternal chromosome expressed.

$X \sim B(n, p)$, where n is the total number of cells of the organism and p is the probability of the cell expressing the paternal chromosome ($p = 0.5$).

By Central Limit Theorem, when $n \geq 30$, number of cells with paternal chromosome expressed can be approximated as $X \sim N(np, np(1-p))$.

Hence, **proportion** of cells with paternal chromosome expressed can be approximated as $\frac{X}{n} \sim N(p, \frac{p(1-p)}{n})$, as we divide expectation by n and variance by n^2. Hence, as n increases, variance decreases.

P37: More Abs

(190 points)

Abscisic acid (ABA) is known to regulate dormancy of seeds and to regulate other stress responses in plants. Phytochromes are light photoreceptors that are involved in photoperception of the light environment. Faith wants to investigate the interactions between phyA and ABI1 and ABI2. She first used a light-switchable Yeast-2-Hybrid (Y2H) system to investigate whether phyA physically interacted with the components in the core ABA signalling pathway, also known as the PYR1–ABI2–OST1–ABF4 pathway (Figure 1A, B). Then she performed firefly luciferase complementation imaging (LCI) assays to investigate phyA and ABI1/2 interactions *in vivo* (Figure 1C, D, E). Finally, to investigate which domain of phyA interacted with ABI1/2, yeast assays were performed using bait vectors expressing different phyA domains fused to LexA DNA-binding domain (Figure 1F, G).

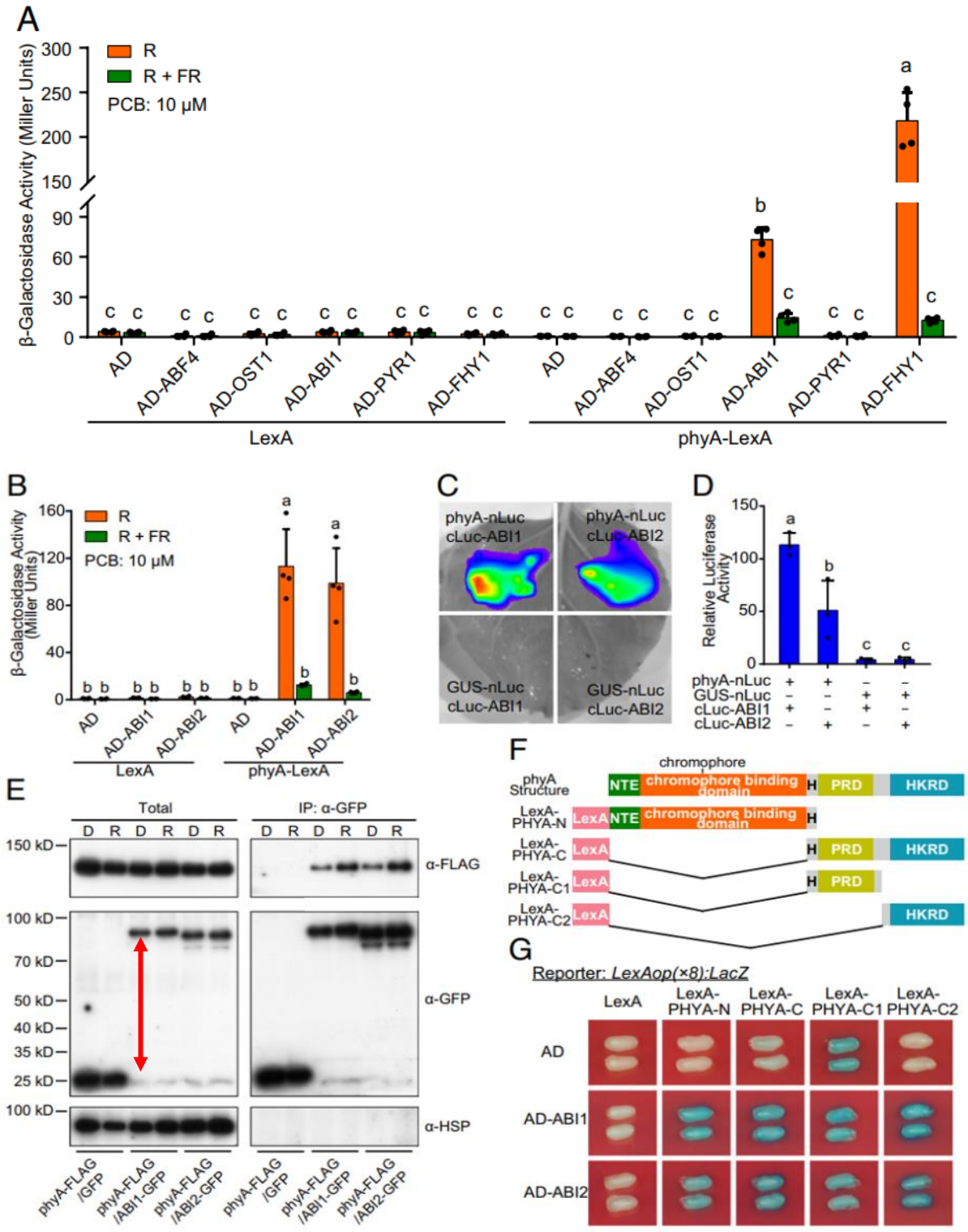


Figure 1: phyA interaction with ABI1 and ABI2. PCB is added as a substrate for phyA. **(A and B)** Y2H assays on phyA interactions with core ABA signalling pathway. **(C and D)** LCI assays on phyA interactions with ABI1/2 in leaves. **(E)** Co-IP assays. Fusion proteins were expressed in protoplasts. D: Kept in dark for 30 min. R: Red light treatment for 30 min. **(F)** Schematic diagram of bait proteins. **(G)** Assays showing ABI1 and ABI2 interactions with different domains of PHYA in the yeast cells.

Q1. Indicate whether the following statements are true or false. **(50 points)**
(Mark each statement as true or false.)

A. AD-FHY1 was used as a negative control in Figure 1A.
B. phyA interacts with OST1 after R light treatment.
C. phyA interacts with ABI1 after R + FR light treatment.
D. phyA does not interact with PYR1 regardless of light treatment.
E. There is no significant difference in interaction between phyA and ABI1 compared to phyA and ABI2 based on Figure 1.

Q2. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. phyA interacts with ABI2 *in vitro* but not *in vivo*.
B. phyA does not interact with ABI1 directly but interacts with ABI1 indirectly.
C. Pr form of phyA interacts with ABI1 preferentially over Pfr form.
D. The red arrow in Figure 1E indicates the molecular weight of phyA-FLAG.

Q3. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The N-terminus domain of phyA can interact with ABI1.
B. The C-terminus domain of phyA can interact with ABI1.
C. PRD does not require ABI1 to activate the PHYA promoter.
D. HKRD does not require ABI2 to activate the PHYA promoter.

In order to further investigate ABA signalling in plant cells, Faith designed a system in yeast cells which emulates the complete ABA-signalling pathway from ABA detection to gene expression of the related genes. The core ABA signalling module contains KF2, SBL1, ABI1, and the downstream transcription factor NON7.

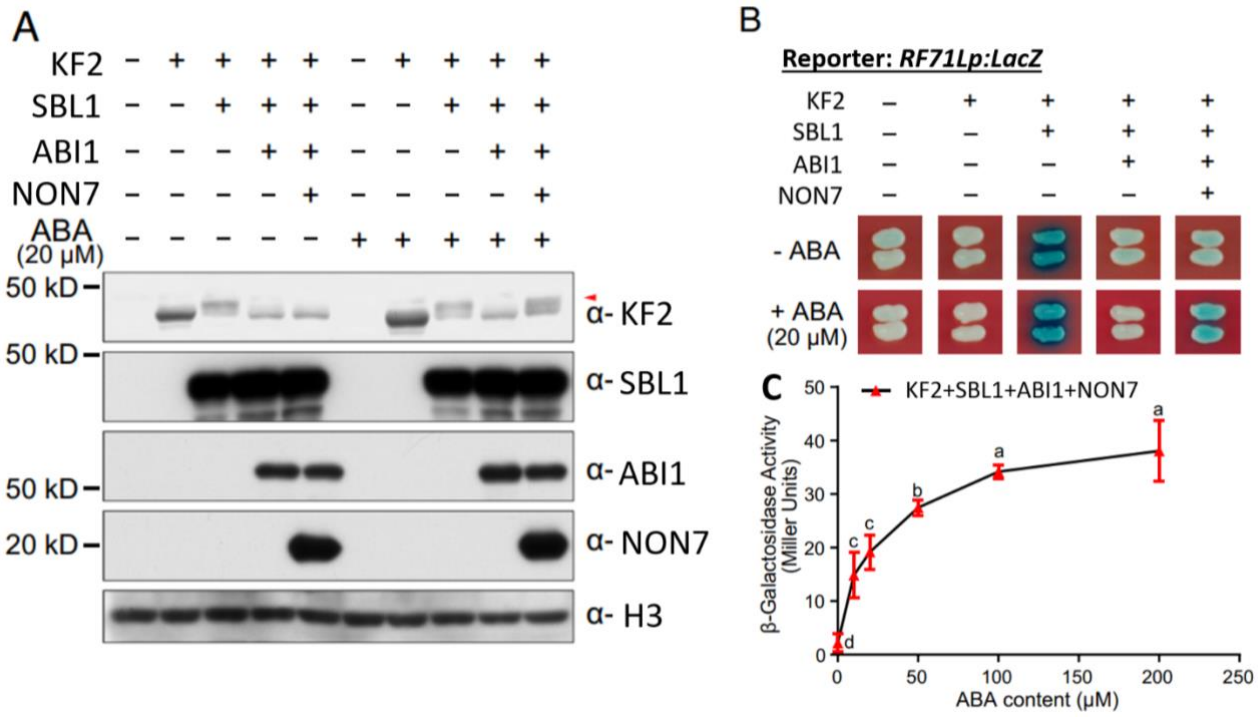


Figure 2: **(A)** Immunoblot assay of transformed yeast cells. The red arrow indicates phosphorylated form of KF2. **(B)** Assay where an ABA-sensitive promoter (*RF71L*) was fused to *LacZ* reporter gene in yeast cells. **(C)** Effects of ABA concentrations on the activation of the *RF71L* promoter in yeast reconstitution system.

Q4. Indicate whether the following statements are true or false. **(50 points)**
(Mark each statement as true or false.)

A. KF2 is an activator protein.
B. SBL1 induces phosphorylation in KF2 which causes transactivation.
C. ABI1 promotes the phosphorylation ability of SBL1.
D. ABI1 inhibits transactivation.
E. Figure 2 supports the hypothesis that NON7 inhibits the effect of ABI1 in the presence of ABA.

Q6. Indicate the approximate minimum concentration of ABA required to induce a saturated activation of the *RF71L* promoter in the presence of KF2+SBL1+ABI1+NON7. **(10 points)**
(Select the correct option.)

A. 0.00mM
B. 0.05mM
C. 0.10mM
D. 0.15mM
E. 0.20mM
F. 0.25mM
G. 0.50mM
H. 1.0mM
I. 15.0mM
J. 25.0mM
K. 50.0mM
L. 100.0mM
M. 150.0mM
N. 200.0mM
O. 250.0mM
P. 500.0mM

Answers and Explanations

Q1.

Answer: **FFFTF**

Explanation:

A. AD-FHY1 is not a component of the core ABA signalling pathway and activity was increased in the presence of R light. Hence it is likely a positive control.
B. No activity implies that there is no interaction.
C. After R + FR light treatment, while there is a slight rise in the activity, we observe that the statistical test indicates that there is no statistical significance between this result and AD (phyA-LexA) as both letters are c. Hence, there is no statistically-significant rise in activity so there is no interaction.
D. Under both conditions, the activity is still zero.
E. While Figure 1B suggests that there is no statistical difference as the activities are the same and the letter is both a, Figure 1D suggests otherwise. With ABI1, the luciferase activity is higher as compared to ABI2, so there is statistical difference in the interactions.

Q2.

Answer: **FFFF**

Explanation:

A. phyA and ABI2 interact *in vitro* according to Figures 1B and 1D. They also interact *in vivo* as seen in Figure 1E as a band is seen. The gel on the right of Figure 1E show that α-GFP was used to immunoprecipitate, thus proteins binding to ABI2-GFP will be obtained. A band is seen at α-FLAG, implying that phyA-FLAG is present, which means phyA and ABI2 both interacted *in vivo* and thus were binding to each other and could be eluted out together.
B. There is direct binding of phyA to ABI1 as a band is seen under α-FLAG.
C. Looking at Figure 1B, interaction between phyA and ABI1 occurred in the presence of R light and not FR light. In the presence of R light, Pr is converted to Pfr, so the Pfr form is more prevalent. Hence, the Pfr form interacts more preferentially with phyA.
D. The gap between the two bands is due to the loss of ABI1. What occurs is that since phyA and ABI1 interact, they form a complex of phyA-FLAG:ABI1-GFP. The antibody against GFP detects it thus forming a band. Hence the complex phyA-FLAG:ABI1-GFP forms the higher band. Meanwhile, GFP is the lower band. phyA-FLAG is not bound because ABI1 is not present to bind to phyA, so phyA will not be able to bind to GFP. Hence, the molecular weight of phyA-FLAG:ABI1 is represented by the red arrow. The statement is hence false because it neglects the molecular weight of ABI1.

Q3.

Answer: **TTTF**

Explanation:

A. Looking at LexA-PHYA-N:AD-ABI1, the yeast cells turned blue from X-gal digestion by β-galactosidase, so there is interaction.
B. Looking at LexA-PHYA-C:AD-ABI1, the yeast cells turned blue from X-gal digestion by β-galactosidase, so there is interaction.
C. In LexA-PHYA-C1:AD, we see that there is autoactivation as this is the negative control and no interaction should occur. Thus, the yeast cells should remain white. This implies that PRD can activate the promoter by itself without the binding of ABI1 or ABI2.
D. Since no autoactivation occurred like what was seen in statement C, the statement is false.

Q4.

Answer: **TTFTT**

Explanation:

A. While not strong enough alone, KF2 is able to activate the *RF71L* promoter in the presence of SBL1, hence turning the yeast cells blue.
B. In Figure 2A, in the presence of SBL1, the phosphorylated form of KF2 is present. This is likely what caused transactivation, which is seen in Figure 2B where the negative control had a positive result (the yeast cells turned blue).
C. The mobility shift is present in Figure 2A when ABI1 is absent, but is absent when ABI1 is present, implying that ABI1 likely inhibits the phosphorylation of KF2. This is also likely why transactivation is inhibited in Figure 2B.
D. In the presence of ABI1, the negative control no longer has a positive result and hence, transactivation is inhibited.
E. In the presence of NON7 and ABA, the yeast cells turned blue implying that it may have inhibited the effect of ABI1 preventing the yeast cells from turning blue.

Q5.

Answer: **C**

Explanation: Figure 2C shows that approximately 100µM of ABA is required to achieve saturation, as at 200µM of ABA there is no significant difference. Hence, the value is likely near 100µM, which is the same as 0.1mM.

Credits

Figures 1 and 2: Li, H., Zhou, Y., Qin, X., Peng, J., Han, R., Lv, Y., Li, C., Qi, L., Qu, G.-P., Yang, L., Li, Y., Terzaghi, W., Li, Z., Qin, F., Gong, Z., Deng, X. W., & Li, J. (2023). Reconstitution of phytochrome A-mediated light modulation of the ABA signaling pathways in yeast. *Proceedings of the National Academy of Sciences*, *120*(34). https://doi.org/10.1073/pnas.2302901120

P38: U + I <3

(210 points)

Urinary tract infections (UTIs) refer to infections of the urinary tract. UTIs caused by kidney stones can lead to damage in the kidneys, where urine is produced. Damaged kidneys can result in a fall in kidney function. A useful way of measuring kidney function is by the glomerular filtration rate (GFR), which measures the rate of filtration of the ultrafiltrate from the Glomerular Capillary (GC) to the Bowman's Space (BS).

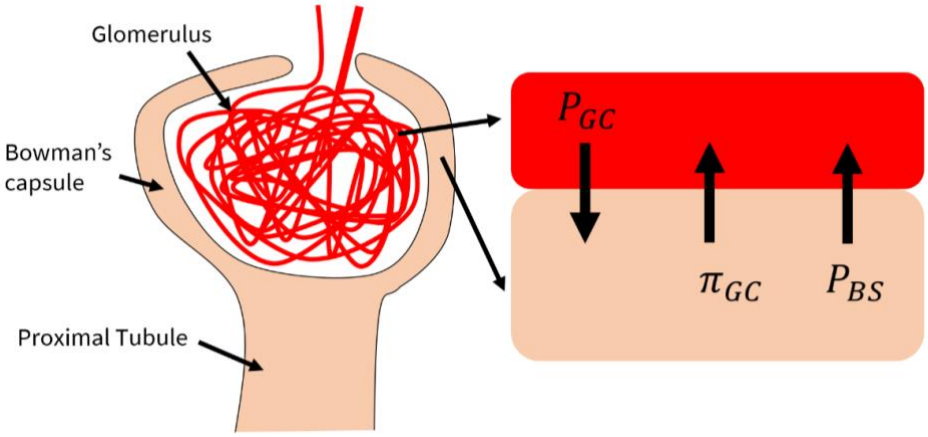


Fig 1: Glomerulus and Bowman's capsule

You may find the following equations may be useful:

$$Net\ glomerular\ filtration\ pressure, P_{net} = P_{GC} - P_{BS} - \pi_{GC}$$

$$Glomerular\ filtration\ rate, GFR = K_f \times P_{net}$$

P refers to the hydrostatic pressure of the fluid, while π refers to the colloidal osmotic pressure. K_f refers to filtration rate.

There are several conditions that can affect the GFR by affecting different factors. Below is a list of possible effects on the GFR and their reason. Use these to answer **Q1** and **Q2**. For the following conditions, indicate whether GFR increases or decreases and the cause.

1. GFR increases because P_{GC} increases.
2. GFR increases because P_{BS} increases.
3. GFR increases because π_{GC} increases.
4. GFR increases because K_f increases.
5. GFR increases because P_{GC} decreases.
6. GFR increases because P_{BS} decreases.
7. GFR increases because π_{GC} decreases.
8. GFR increases because K_f decreases.
9. GFR decreases because P_{GC} increases.
10. GFR decreases because P_{BS} increases.
11. GFR decreases because π_{GC} increases.
12. GFR decreases because K_f increases.
13. GFR decreases because P_{GC} decreases.
14. GFR decreases because P_{BS} decreases.
15. GFR decreases because π_{GC} decreases.
16. GFR decreases because K_f decreases.

Q1. Match the correct effects on GFR of the following conditions and their corresponding reason (1-16) **(30 points)**

(Match the correct number to the correct row.)

Condition	**Effect on GFR (1-16)**
Diabetes mellitus	
Adrenaline rush	
Blockage in tubular lumen by uric acid crystal	

Q2. Match the correct effects on GFR of the following conditions and their corresponding reason (1-16) **(30 points)**

(Match the correct number to the correct row.)

Condition	**Effect on GFR (1-16)**
Acute hypertension	
Acute heart failure	
Increased albumin synthesis by liver	

A useful way of quantifying renal function is in terms of clearance. The renal clearance of any substance is the volume of plasma from which the substance is completely removed ("cleared") by the kidneys per unit time. The concept is based on the conservation of mass, where any substance that is cleared from the blood by the kidneys must be found in the urine.

$$C_S = \frac{U_S \times V}{P_S}$$

Where C_S is clearance of S, U_S is urine concentration of S, V is urine flow rate and P_S is plasma concentration of urine.

Q3. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. The renal clearance of glucose of a patient with diabetes mellitus is greater than a normal, healthy human.
B. A substance that is freely filtered, but neither secreted nor reabsorbed, can be used to measure the renal plasma flow.
C. Renal clearance is always less than GFR.
D. Assuming all other factors are kept constant, an increase in secretion of ADH by the posterior pituitary results in a change in clearance of most substances.

The osmolarity of the intracellular fluids and extracellular fluids can be affected by different conditions. Figure 2 shows the graph of osmolarity against volume of the intracellular and extracellular fluids of a normal person.

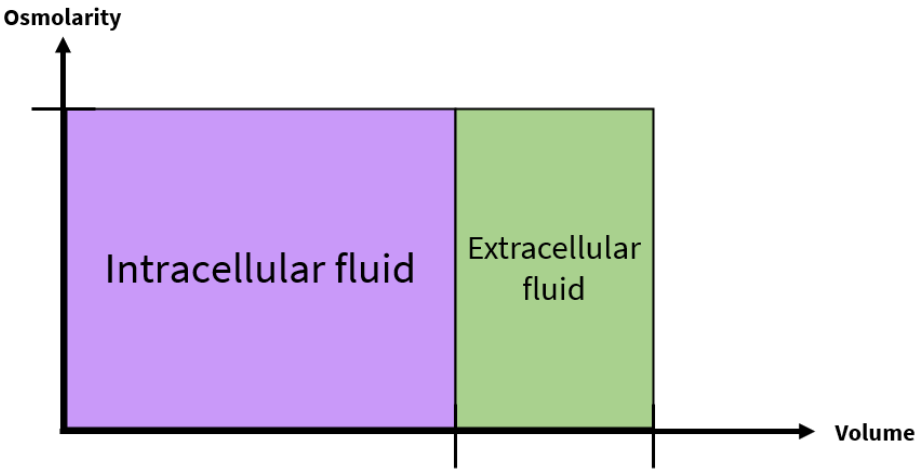


Figure 2: Graph of osmolarity against volume of the intracellular and extracellular fluids of a normal person. Axes markers have been indicated to indicate the values of the x and y-axes for the normal person.

Figure 3 shows possible effects on the graph in Figure 2 due to changes in conditions.

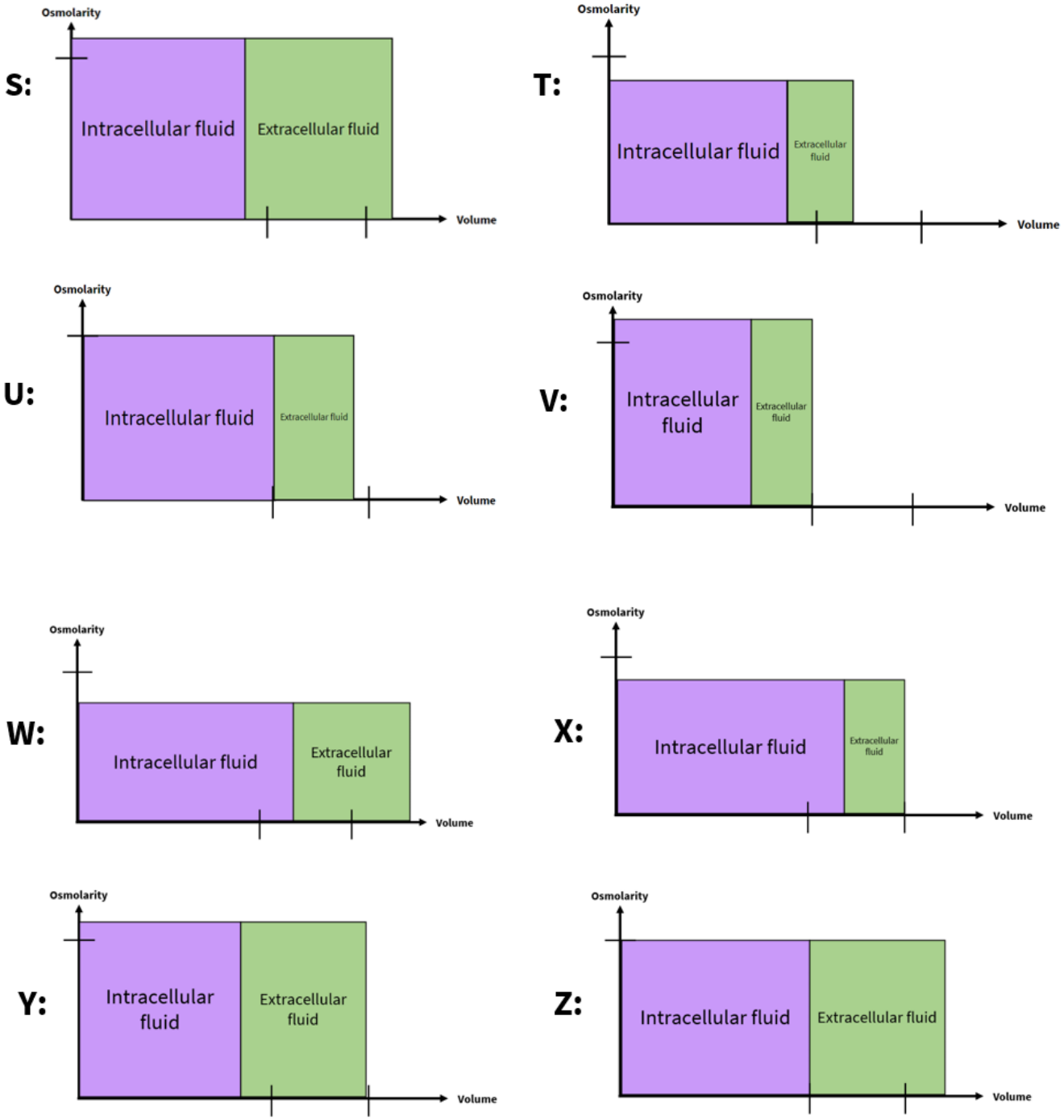


Figure 3: Possible changes in graph. The axes markers indicate the values of the x and y-axes for the normal person (Figure 2).

Q4. Match the following solutions to the correct effect on the graph (S-Z) when NaCl solutions with varying concentrations are ingested. If there is no change, enter *No change*. **(30 points)**
(Match the correct letter to the correct row.)

Solution added	**Graph (U-Z)**
3% NaCl saline	
0.9% NaCl saline	
0.3% NaCl saline	

Q5. Certain conditions can also result in the changes seen in Figure 3. Match the following conditions to the correct effect of it on the graph (S-Z). If there is no change, enter *No change*. If the graph is not in Figure 3, enter None. **(40 points)**
(Match the correct letter to the correct row.)

Condition	**Graph (U-Z)**
Hypersecretion of ADH	
Primary hyperaldosteronism	
Right after eating potato chips	
Inability of kidney to respond to ADH	

Bartter's Syndrome is a rare disorder involving the kidneys. It occurs due to a rare autosomal-recessive allele that impairs the function of the 1-sodium, 2-chloride, 1-potassium cotransporter (NKCC), which is responsible for the active transport of solutes from the tubular lumen to the medullary interstitial fluid in the ascending loop of Henle.

Q6. Determine if the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. If both parents do not have Barrter's Syndrome, it is impossible for any of their children to have Barrter's Syndrome (assuming no mutations).
B. The hyperosmotic medullary interstitial fluid will become less hyperosmotic relative to the rest of the body.
C. The ability of the kidneys to excrete hyperosmotic urine will be reduced.
D. Furosemide, an NKCC inhibitor, can treat Barrter's Syndrome.

Answers and Explanations

Q1.

Answer: **16, 13, 10**

Explanation:

A. Diabetes mellitus leads to destruction of the kidney and the decrease in number of functional nephrons, leading to a lower K_f and hence a lower GFR.
B. Adrenaline leads to vasoconstriction of the afferent arteriole, leading to a lower P_{GC} and hence a lower GFR.
C. Blockage in tubular lumen by uric acid crystal leads to an accumulation of filtrate in the tubules, leading to a higher P_{BS} and hence a lower GFR.

Q1.

Answer: **1, 13, 11**

Explanation:

A. Hypertension leads to a rise in P_{GC} and hence a higher GFR.
B. Acute heart failure leads to a lower cardiac output and hence mean arterial blood pressure and hence a lower P_{GC} and GFR.
C. Increased albumin synthesis by liver leads to increased plasma colloidal osmotic pressure and hence a higher π_{GC} and hence lower GFR.

Q3.

Answer: **TFFF**

A. In a normal human, there should be no glucose in the urine so renal clearance of glucose is 0. In patients with diabetes mellitus, glycosuria results so renal clearance of glucose is non-zero.
B. A substance that is freely filtered, but neither secreted nor reabsorbed, is used to measure glomerular filtration rate. Renal plasma flow is measured by a substance that is fully secreted.
C. Renal clearance of a substance can be higher than glomerular filtration rate if the rate of secretion exceeds the rate of reabsorption.
D. The clearance of most substances will remain unchanged. An increase in secretion of ADH will result in a decrease in urine flow rate but a proportional increase is urine concentration of the

substance (i.e. concentration of substance and volume of urine changes, but amount of solutes remain the same).

Q4.

Answer: **S, Z, W**

A. Solution is hypertonic with respect to plasma, so results in an increase in osmolarity of intra and extracellular fluid. There is an increase in total volume of the body, with a notable increase in extracellular fluid and decrease in intracellular fluid, as NaCl in the extracellular fluid cause water to leave the cell by osmosis.
B. Solution is isotonic with respect to plasma, hence osmolarity remains unchanged. There is an increase in total volume of the body.
C. Solution is hypotonic with respect to plasma, so results in a decrease in osmolarity of intra and extracellular fluid. There is an increase in total volume of the body, with a notable increase in intracellular fluid and decrease in extracellular fluid, as excess solutes in the intracellular fluid cause water to enter the cell by osmosis.

Q5.

Answer: **W, Z, Y, V**

A. Hypersecretion of ADH will cause retention of water, causing osmolarity to fall and total volume to rise. There is a notable increase in intracellular fluid and decrease in extracellular fluid, as excess solutes in the intracellular fluid cause water to enter the cell by osmosis.
B. Hyperaldosteronism leads to increased retention of both Na^+, causing water to follow by osmosis. Hence, total volume increased, while osmolarity remains unchanged.
C. Osmolarity increases due to salt intake, with no corresponding water intake. Total volume remains unchanged as there is minimal water in potato chips.
D. This will cause polyuria, where there is excessive production of dilute urine, causing total volume to fall. As hypotonic urine is excreted, a greater proportion of water is lost compared to solutes, resulting in an increase in osmolarity.

Q6.

Answer: **FTTF**

A. If both parents are heterozygous for the mutation, it is possible for a child to inherit both mutations, one from each parent, hence becoming homozygous recessive for the mutation and expressing Barrter's Syndrome.
B. Without the NKCC pump, minimal Na^+, K^+ and Cl^- will be pumped into the medullary interstitial fluid, resulting in an inability to accumulate ions and hence becomes less hyperosmotic relative to the rest of the body.
C. With the medulla becoming less hyperosmotic, the medulla is less able to absorb water from the filtrate in the tubular lumen in the Loop of Henle and collecting duct, and hence the ability of the nephrons to concentrate the filtrate is reduced. Hence, the urine produced will be less hyperosmotic.
D. Furosemide blocks NKCC, which is already non-functional in patients with Barrter's Syndrome, so there will be no observable effects.

P39: Heat. Cool. Multiply.

(220 points)

Figure 1 shows the stages of a polymerase chain reaction (PCR).

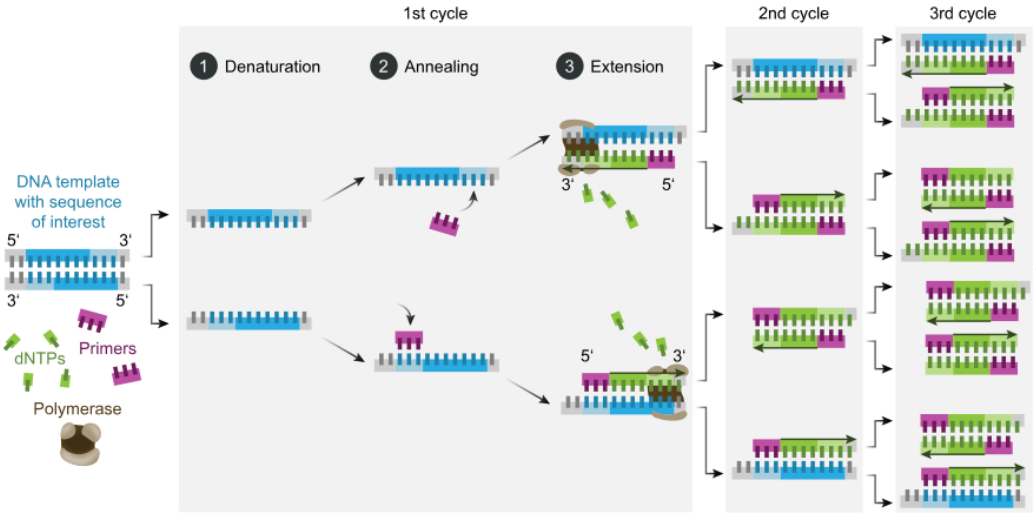


Figure 1: Schematic diagram of stages of polymerase chain reaction (PCR)

Dr Ace carries out a PCR reaction to amplify a region of interest, starting with 10 nmol of genomic DNA. Dr Ace is very fastidious about the product obtained from the PCR. He wants to know exactly how much product with the correct strand length he will obtain in each cycle. This means that his definition of "product" is a dsDNA molecule with the exact length of the region of interest.

We first assume no mutations in the PCR.

Q1. How many nanomoles of product (dsDNA with the correct strand length) does he have after 1 PCR cycle? **(10 points)**
(Enter your answer correct to 3 s.f. Do not include any units.)

Q2. How many nanomoles of product (dsDNA with the correct strand length) does he have after 2 PCR cycles? **(10 points)**
(Enter your answer correct to 3 s.f. Do not include any units.)

Q3. How many nanomoles of product (dsDNA with the correct strand length) does he have after 3 PCR cycles? **(10 points)**
(Enter your answer correct to 3 s.f. Do not include any units.)

Q4. How many **micromoles** of product (dsDNA with the correct strand length) does he have after 15 PCR cycles? **(30 points)**
(Enter your answer correct to 3 s.f. Do not include any units.)

Now, mutations are not negligible and have to be accounted for. Assume that the rate of error in DNA replication by *Taq* polymerase is 1 error per 10000 nucleotides. Given that the length of the region of interest is 220 base pairs and the reverse and forward primers are 20 base pairs each (assume no mutation in primers), help Dr Ace figure out how much product he will obtain below.

> **Q5**. How many nanomoles of product (dsDNA with the correct strand length with no mutations) does he have after 3 PCR cycles? **(30 points)**
> *(Enter your answer correct to 3 s.f. Do not include any units.)*
>
> **Q6**. What is the minimum number of cycles required to get 1 mmol of product (dsDNA with the correct strand length with no mutations)? *(Hint: the use of Excel may be very helpful.)* **(40 points)**
> *(Enter your answer correct to 3 s.f. Do not include any units.)*

Dr Ace is annoyed by the changes in the calculation required when mutations are taken into account. Hence, he decided that from now on, we can assume that there are no mutations unless otherwise stated.

Dr Ace wants to amplify a 400-bp gene of interest found in the mitochondria of blue fiddler crabs (*Tubuca paradussumieri*). He decides to get creative and decides to perform some modifications on his PCR protocol to investigate whether PCR will still be carried out normally and the desired product is obtained.

Dr Ace's original protocol along with four other protocols with modifications are shown below.

Original protocol: Dr Ace mixes the linear gene of interest in an Eppendorf tube with a large excess of 20-bp primers (>100x of final expected dsDNA products) and adds buffer mix. He then adds excess dNTPs and *Taq* polymerase. Dr Ace then inverts the tube multiple times and places it in a thermocycler set at 35 PCR cycles of *1. 95°C / 2. 60°C / 3. 72°C*. After the last cycle, the mixture is immediately cooled to 15°C and the desired dsDNA products are collected.

Results: *n* moles of product were obtained.

Protocol	**Modifications**
Protocol 1	Dr Ace changed the order of the thermocycle from *1. 95°C / 2. 60°C / 3. 72°C* to *1. 60°C / 2. 72°C / 3. 95°C*.
Protocol 2	Dr Ace used 60-bp primers instead of 20-bp primers. The annealing temperature is optimal based on the melting temperatures of the two 60-bp primers.
Protocol 3	Dr Ace added DNA helicase (and relevant cofactors and accessory proteins like single-strand DNA-binding proteins (SSB)) to the Eppendorf tube. He did not use the

	thermocycler but instead left the mixture to incubate for 4h at 37°C. He subsequently then collected the desired product.
Protocol 4	Dr Ace added two 20-bp primers (in excess) to the Eppendorf tube. These primers anneal to the centre of the gene of interest (See Figure 2).
Protocol 5	Dr Ace used a more heat-sensitive polymerase (Polymerase *K*) than *Taq* polymerase instead.

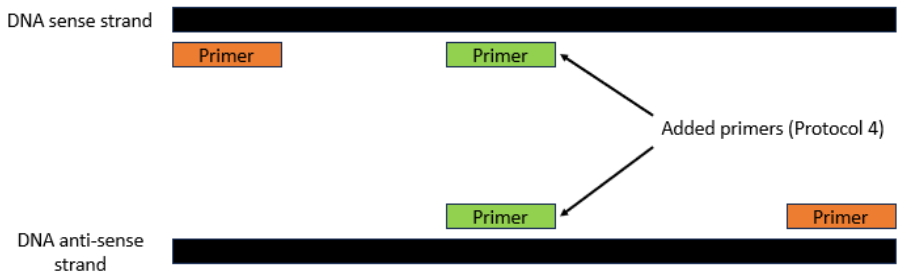


Figure 2: Protocol 4 Primers

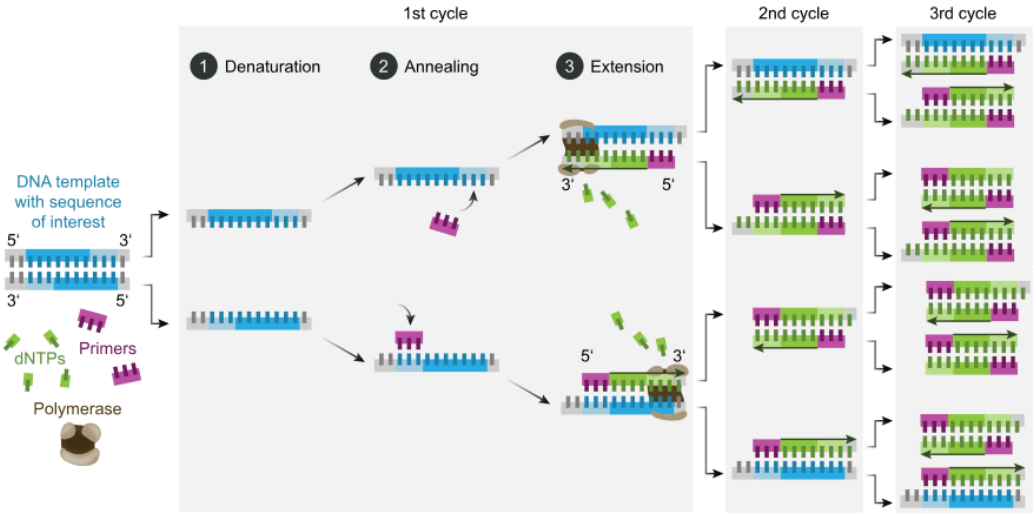
Q7. Indicate whether the following statements are true or false. **(50 points)**
(Mark each statement as true or false.)

A. PCR has rendered gene cloning by transformation of *E. coli* relatively obsolete.
B. The product must be circularised before it can be used for further analysis (such as Southern Blot).
C. *Taq* polymerase synthesises dsRNA to amplify mRNA in cells to measure gene expression levels.
D. The amount of product obtained in Protocol 1 will be significantly reduced as compared to the original protocol.
E. Assuming mutations are not negligible, a higher proportion of the products obtained in Protocol 2 will have mutations as compared to the original protocol.

Q8. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. Dr Ace would likely still be able to obtain his desired product in Protocol 3.
B. Polymerase *K* likely has more cysteine residues than *Taq* polymerase.
C. A similar amount of product will be obtained in Protocol 4 as compared to the original protocol.
D. A lower amount of product will be obtained in Protocol 5 as compared to the original protocol.

Answers and Explanations



Analysing the PCR process

From the diagram, you can tell that there are 3 types of DNA strands: overextended on both sides (BO), overextended on one side (SO) and correct length (CL). We introduce the concept of a *single-stranded (ss) derivative* strand. The template strands (BO) in the initial PCR mixture are 0^{th}-derivative strand, the daughter strands synthesised by using the 0^{th}-derivative strand as template strand are 1^{st}-derivative strands (SO). We can generalise this by saying that the daughter strands synthesised by using the n^{th}-derivative strand as template strand are $n+1^{th}$-derivative strands.

Assuming no mutations, the number of n^{th}-derivative strands in the k^{th} cycle, $u_{n,k} = u_{n,k-1} + u_{n-1,k-1}$.

We notice that 2^{nd}-derivative strands and any higher-order strands are all of correct length. Hence, the amount of product (*dsDNA* with the correct strand length) in the k^{th} cycle is the amount of n^{th}-derivative strands in the $k-1^{th}$ cycle, where $n \geq 2$. Note that 10 nmol of DNA corresponds to 1 au of dsDNA and 2 au of ssDNA.

Plotting, on Excel, we solve Q1 to Q4.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
1			Cycle																									
2			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
3	Number of ss strands (derivative)	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
4		1	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50
5		2	0	0	2	6	12	20	30	42	56	72	90	110	132	156	182	210	240	272	306	342	380	420	462	506	552	600
6		3	0	0	0	2	8	20	40	70	112	168	240	330	440	572	728	910	1120	1360	1632	1938	2280	2660	3080	3542	4048	4600
7		4	0	0	0	0	2	10	30	70	140	252	420	660	990	1430	2002	2730	3640	4760	6120	7752	9690	11970	14630	17710	21252	25300
8		5	0	0	0	0	0	2	12	42	112	252	504	924	1584	2574	4004	6006	8736	12376	17136	23256	31008	40698	52668	67298	85008	106260
9		6	0	0	0	0	0	0	2	14	56	168	420	924	1848	3432	6006	10010	16016	24752	37128	54264	77520	108528	149226	201894	269192	354200
10		7	0	0	0	0	0	0	0	2	16	72	240	660	1584	3432	6864	12870	22880	38896	63648	100776	155040	232560	341088	490314	692208	961400
11		8	0	0	0	0	0	0	0	0	2	18	90	330	990	2574	6006	12870	25740	48620	87516	151164	251940	406980	639540	980628	1470942	2163150
12		9	0	0	0	0	0	0	0	0	0	2	20	110	440	1430	4004	10010	22880	48620	97240	184756	335920	587860	994840	1634380	2615008	4085950
13		10	0	0	0	0	0	0	0	0	0	0	2	22	132	572	2002	6006	16016	38896	87516	184756	369512	705432	1293292	2288132	3922512	6537520
14		11	0	0	0	0	0	0	0	0	0	0	0	2	24	156	728	2730	8736	24752	63648	151164	335920	705432	1410864	2704156	4992288	8914800
15		12	0	0	0	0	0	0	0	0	0	0	0	0	2	26	182	910	3640	12376	37128	100776	251940	587860	1293292	2704156	5408312	1E+07
16		13	0	0	0	0	0	0	0	0	0	0	0	0	0	2	28	210	1120	4760	17136	54264	155040	406980	994840	2288132	4992288	1E+07
17		14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	30	240	1360	6120	23256	77520	232560	639540	1634380	3922512	8914800
18		15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	32	272	1632	7752	31008	108528	341088	980628	2615008	6537520
19		16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	34	306	1938	9690	40698	149226	490314	1470942	4085950
20		17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	36	342	2280	11970	52668	201894	692208	2163150
21		18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	38	380	2660	14630	67298	269192	961400
22		19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	40	420	3080	17710	85008	354200
23		20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	42	462	3542	21252	106260
24	Total number of ss strands of correct length		0	0	2	8	22	52	114	240	494	1004	2026	4072	8166	16356	32738	65504	131038	262108	524250	1048536	2097110	4194258	8388516	1.7E+07	3.4E+07	6.7E+07
25	Total number of ds strands of correct length		0	0	0	2	8	22	52	114	240	494	1004	2026	4072	8166	16356	32738	65504	131038	262108	524250	1048536	2097110	4194258	8388516	1.7E+07	3.4E+07
26	Amount of ds strands of correct length / nmol		0	0	0	20	80	220	520	1140	2400	4940	10040	20260	40720	81660	163560	327380	655040	1310380	2621080	5242500	1E+07	2.1E+07	4.2E+07	8.4E+07	1.7E+08	3.4E+08

Alternatively, we can realise that there will always be 2 ss 0^{th}-derivative strand and $2k-2$ ss 1^{st}-derivative strand in the $k-1^{th}$ cycle out of a total of 2^k ss strands in the $k-1^{th}$ cycle. This means that there are $2^k-(2k-2)-2=2^k-2k$ ss strands in the $k-1$ cycle of 2^{nd}-derivative or higher, which will give rise to 2^k-2k ds strands of correct length in the k^{th} cycle of replication. Hence, the number of ds strands of the correct length is $10(2^k-2k)$.

Q1.

Answer: **0**

Explanation: As explained above.

Q2.

Answer: **0**

Explanation: As explained above.

Q3.

Answer: **20**

Explanation: As explained above.

Q4.

Answer: **327**

Explanation: As explained above.

Analysing the mutations in PCR

First, calculate the probability of a strand being perfectly synthesised.

Ideally, Probability of no incorrect base pairs per strand synthesised$= (1 - 0.0001)^{200}$, but we can approximate as the following as 0.0001 is so small.

Probability of no incorrect base pair per strand synthesised$= 1 - (200 * 0.0001) = 0.98$

Hence, the number of n^{th}-derivative strands in the k^{th} cycle, $u_{n,k} = u_{n,k-1} + 0.98u_{n-1,k-1}$

Then, the amount of product (*dsDNA* with the correct strand length) in the k^{th} cycle is 0.98 of the amount of n^{th}-derivative strands in the $k - 1^{th}$ cycle, where $n \geq 2$. Note that 10 nmol of DNA corresponds to 1 au of dsDNA and 2 au of ssDNA.

Plotting on Excel:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
1			Cycle																									
2			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
3	Number of ss strands (derivative)	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
4		1	0	1.96	3.92	5.88	7.84	9.8	11.76	13.72	15.68	17.64	19.6	21.56	23.52	25.48	27.44	29.4	31.36	33.32	35.28	37.24	39.2	41.16	43.12	45.08	47.04	49
5		2	0	0	1.9208	5.7624	11.5248	19.208	28.812	40.3368	53.7824	69.1488	86.436	105.644	126.773	149.822	174.793	201.684	230.496	261.229	293.882	328.457	364.952	403.368	443.705	485.962	530.141	576.24
6		3	0	0	0	1.88238	7.52954	18.8238	37.6477	65.8834	105.414	158.12	225.886	310.593	414.124	538.362	685.188	856.485	1054.14	1280.02	1536.03	1824.03	2145.92	2503.57	2898.87	3333.7	3809.95	4329.48
7		4	0	0	0	0	1.84474	9.22368	27.671	64.5658	129.132	232.437	387.395	608.763	913.144	1318.99	1846.58	2518.07	3357.42	4390.47	5644.89	7150.2	8937.75	11040.7	13494.2	16335.1	19602.2	23335.9
8		5	0	0	0	0	0	1.80784	10.847	37.9647	101.239	227.788	455.576	835.223	1431.81	2326.69	3619.3	5428.95	7896.65	11186.9	15489.6	21021.6	28028.8	36787.8	47607.7	60832.1	76840.5	96050.6
9		6	0	0	0	0	0	0	1.77168	12.4018	49.6072	148.822	372.054	818.518	1637.04	3040.21	5320.37	8867.28	14187.7	21926.4	32889.6	48069.4	68670.5	96138.7	132191	178846	238462	313765
10		7	0	0	0	0	0	0	0	1.73625	13.89	62.505	208.35	572.963	1375.11	2979.41	5958.81	11172.8	19862.7	33766.6	55254.5	87486.2	134594	201891	296107	425654	600923	834616
11		8	0	0	0	0	0	0	0	0	1.70153	15.3137	76.5687	280.752	842.255	2189.86	5109.68	10949.3	21898.6	41364.1	74455.4	128605	214341	346244	544097	834282	1251423	1840328
12		9	0	0	0	0	0	0	0	0	0	1.6675	16.675	91.7123	366.849	1192.26	3338.33	8345.82	19076.1	40536.8	81073.6	154040	280073	490127	829446	1362661	2180257	3406652
13		10	0	0	0	0	0	0	0	0	0	0	1.63415	17.9756	107.854	467.366	1635.78	4907.34	13086.2	31780.9	71506.9	150959	301918	576389	1056714	1869570	3204978	5341630
14		11	0	0	0	0	0	0	0	0	0	0	0	1.60146	19.2176	124.914	582.932	2186	6995.19	19819.7	50964.9	121042	268982	564862	1129723	2165302	3997482	7138360
15		12	0	0	0	0	0	0	0	0	0	0	0	0	1.56943	20.4026	142.818	714.092	2856.37	9711.65	29135	79080.6	197702	461304	1014868	2121996	4243993	8161525
16		13	0	0	0	0	0	0	0	0	0	0	0	0	0	1.53804	21.5326	161.495	861.305	3660.55	13178	41730.2	119229	312977	765054	1759625	3839181	7998294
17		14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.50728	22.6093	180.874	1024.95	4612.29	17526.7	58422.3	175267	481984	1231737	2956170	6718567
18		15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.47714	23.6342	200.891	1205.34	5725.39	22901.6	80155.4	251917	724262	1931364	4828410
19		16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.4476	24.6091	221.482	1402.72	7013.6	29457.1	108009	354888	1064664	2957401
20		17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.41864	25.5356	242.588	1617.25	8490.58	37358.6	143208	490998	1534369
21		18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.39027	26.4151	264.151	1849.06	10169.8	46781.2	187125	668303
22		19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.36247	27.2493	286.118	2098.2	12064.6	57910.2	241293
23		20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	Total number of ss strands of correct length		0	0	1.9208	7.64478	20.8991	49.0634	106.749	222.889	454.765	915.802	1830.57	3643.75	7235.74	14349.8	28437.6	56333.4	111569	220937	437488	866261	1715233	3396172	6724181	1.3E+07	2.6E+07	5.2E+07
25	Total number of ds strands of correct length		0	0	0	1.88238	7.49189	20.4811	48.0821	104.614	218.431	445.67	897.486	1793.96	3570.87	7091.03	14062.8	27868.9	55206.7	109338	216518	428739	848936	1680928	3328249	6589698	1.3E+07	2.6E+07
26	Amount of ds strands of correct length / nmol		0	0	0	18.8238	74.9189	204.811	480.821	1046.14	2184.31	4456.7	8974.86	17939.6	35708.7	70910.3	140628	278689	552067	1093375	2165184	4287385	8489361	1.7E+07	3.3E+07	6.6E+07	1.3E+08	2.6E+08

Alternatively, we can realise that each cycle of replication multiplies the total number of ss strands by $1 + 0.98 = 1.98$. Hence, $u_k = 1.98u_{k-1}$, where u_k is the number of ss strands in the k^{th} cycle.

So total number of ss strands in the k^{th} cycle, $u_k = 2(1.98)^k$

Total number of 0^{th}-derivative strand will always remain as 2.

Number of 1^{st}-derivative strand synthesised in each cycle is $2 * 0.98 = 1.96$

So total number of 1^{st}-derivative strand synthesised in k^{th} is $1.96k$

Hence, total number of ss strands of 2^{nd}-derivative or higher in the $k - 1^{th}$ cycle $= 2(1.98)^{k-1} - 2 - 1.96(k - 1)$

Those strands will act as templates to give the total number of ds strands of the correct length in the k^{th} cycle, so the total number of correct product is 0.98 times of the equation above.

Hence, total amount of correct product in the k^{th} cycle $= 10 * 0.98(2(1.98)^{k-1} - 2 - 1.96(k - 1))$

Q5.

Answer: **18.8**

Explanation: As explained above.

Q6.

Answer: **17**

Explanation: As explained above.

Q7.

Answer: **FFFTF**

A. As this question would have taught you, fidelity of replication by PCR is low, so it will not be suitable to produce many copies of long strands of DNA.
B. DNA can be in linear form during analysis by Southern blot (in fact it usually is).
C. mRNA is first reverse transcribed into cDNA, before being amplified by *Taq* polymerase.
D. At first glance, it may seem that this modification has little impact on the overall result and that this only reduces the number of cycles by one. This is true for the middle cycles, where PCR occurs as per normal. However, it is imperative to examine the final step. At the final step: 1) 60°C allows DNA to anneal to primer; 2) 72°C allows extension by *Taq* polymerase; 3) 90°C leads to denaturation to ssDNA. When the product is cooled to 30°C for collection, the ssDNA anneals to primers as excess primers are present in PCR reactions, hence little to none of the required dsDNA will be found. Very low amounts of the complementary full ssDNA strands will anneal together to form the required dsDNA product because the primers are in excess by >100x.
E. A lower proportion will be mutated as there are fewer nucleotides synthesised per strand if the primer is longer.

Q8.

Answer: **TFFT** (Take a look at the organising team's About Us page! 😊)

A. This is known as helicase-dependent isothermal DNA amplification. You can read more about it in the following article: Vincent M., Xu Y., & Kong H. (2004). *Helicase-dependent isothermal DNA amplification*. EMBO Reports. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1249482/
B. Since polymerase *K* is heat-sensitive, it likely has fewer covalent disulfide bridges holding its tertiary structure together.
C. The additional primers will result in the formation of shorter products, resulting in products of the desired length being formed.
D. Since polymerase *K* is heat-sensitive, it is likely to denature earlier and hence result in less replication of the DNA sequence.

Credits

Figure 1: Enzoklop. (2020, November 12) *Polymerase chain reaction*. Wikipedia. https://en.wikipedia.org/wiki/Polymerase_chain_reaction#/media/File:Polymerase_chain_reaction-en.svg

Protocol 3 Content reference: Vincent M., Xu Y., & Kong H. (2004). *Helicase-dependent isothermal DNA amplification*. EMBO Reports. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1249482/

P40: Anaemia

(180 points)

The Intriguing Case of Thalassaemias

Thalassaemias are a type of anaemia found in humans. The underlying pathophysiology of the thalassaemias involves an imbalance in the synthesis of haemoglobin alpha (α-) and beta (β-) globins. Owing to deficiency in either globin chain, the other chain is in excess, causing the unpaired chains to precipitate as insoluble inclusions.

Due to gene duplication, humans have two α-globin genes on chromosome 16, making for a total of four of these genes in the diploid genome. α-thalassaemia trait refers to deletion of two out of four of these genes. Both deletions may occur on the same chromosome (more common in population X) or one deletion on each chromosome (more common in African population Y). Clinically significant α-thalassaemia develops when three or more of these genes are deleted.

Q1. Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. Persons with β-thalassaemia are likely to have an excess of the form of bilirubin that is soluble in water.
B. Mutations that create new splice sites are likely to result in a reduction in β-globin synthesis rather than an absence of β-globin synthesis.
C. Persons with deletions in both α- and β-globin genes are likely to have more severe symptoms than those with an equivalent number of deletions but in the α- or β-globin genes only.
D. Clinically significant α-thalassaemia is likely to be more common in population X than population Y.

Types of Anaemia

Anaemia refers to a low level of red blood cells (RBCs). The following table shows several types of anaemia.

Code	Type of Anaemia	Description
I	Hereditary spherocytosis	Mutations in proteins e.g. ankyrin, spectrin tethering cytoskeleton to plasma membrane causes RBCs to shed membrane fragments, becoming spheroid, less deformable and vulnerable to splenic destruction
II	Glucose-6-phosphate dehydrogenase (G6PD) deficiency	X-linked recessive disorder in which lack of G6PD renders RBCs susceptible to oxidant stress
III	Sickle cell anaemia	Mutation in haemoglobin beta globin resulting in formation of rigid fibres that cause RBCs to become sickle-shaped. May lead to autosplenectomy.
IV	Beta thalassaemia	Mutations resulting in absent or reduced haemoglobin beta globin synthesis
V	Alpha thalassaemia	Mutations resulting in absent or reduced haemoglobin alpha globin synthesis
VI	Paroxysmal nocturnal haemoglobinuria	X-linked mutation in phosphatidylinositol glycan complementation group A (PIGA) gene resulting in deficiency of glycosylphosphatidylinositol (GPI) anchored proteins. Due to increase in complement activity when blood pH falls during sleep.
VII	Immunohaemolytic anaemia	Caused by antibodies binding to RBCs
VIII	Pernicious anaemia	Caused by deficiency in vitamin B12 required for erythropoiesis
IX	Folate-deficiency anaemia	Caused by deficiency in folate required for erythropoiesis
X	Iron-deficiency anaemia	Results from dietary lack of iron or chronic blood loss

The normal laboratory values for several haematologic measurements are shown below.

Measurement	**Reference range**
Haematocrit	Male: 41-50% | Female: 36-46%
Haemoglobin	Male: 13.5-16.5 g/dL | Female: 12.0-15.0 g/dL
Mean corpuscular haemoglobin (MCH)	26-34 pg/cell
Mean corpuscular volume (MCV)	80-100 μm^3

For **Q2** to **Q8**, while the information provided is not sufficient to establish a definitive diagnosis, it is known that each patient suffers from one of the anaemic conditions listed above. Use the given history of the patient to help deduce from which form of anaemia each patient is likely to suffer.

Q2. A man complains of nausea and vomiting in the past few months. Laboratory findings show haematocrit of 38%, haemoglobin of 12.8 g/dL, MCH of 37 pg/cell, and MCV of 120μm^3. His gastric pH is higher than normal, but he denies taking any proton pump inhibitors recently. Endoscopic biopsy reveals degeneration of gastric glands in the stomach mucosa. Urease breath test is negative. Antibodies against parietal cells are detected in the plasma. Enter the code (I-X) corresponding to the type of anaemia which most likely accounts for these results. **(20 points)**
(Enter a roman numeral.)

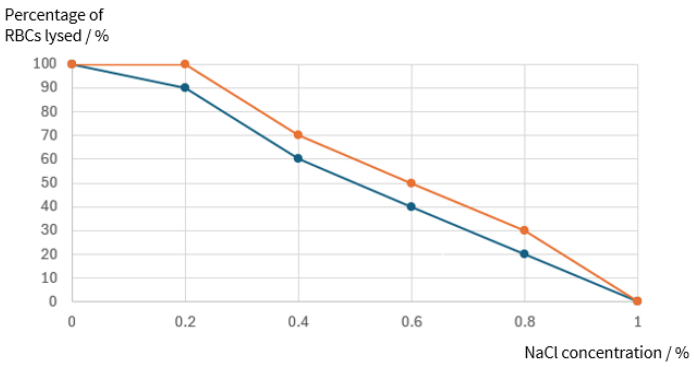


Figure 1: Percentage of RBCs lysed at different NaCl concentrations. Blue: Normal, Orange: Patient

Q3. A woman presents to the clinic with a pale appearance. She has haematocrit of 34% and MCV of 90μm^3. When her RBCs are mixed with antibodies specific for immunoglobulin, no agglutination occurs. Figure 1 shows the percentage of RBCs lysed at different NaCl concentrations. Enter the code (I-X) corresponding to the type of anaemia which most likely accounts for these results. **(20 points)**
(Enter a roman numeral.)

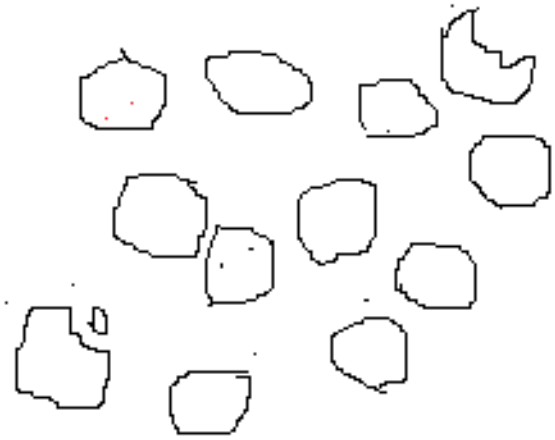


Figure 2: Peripheral blood smear

Q4. An African boy presents to the clinic with malaise and jaundice. He has haematocrit of 34% and MCV of 90µm^3. His spleen is normal on examination. When his RBCs are mixed with antibodies specific for immunoglobulin, no agglutination occurs. He recently contracted malaria, but recovered after treatment was given. Figure 2 shows a representative drawing of his peripheral blood smear. Enter the code (I-X) corresponding to the type of anaemia which most likely accounts for these results. **(20 points)**
(Enter a roman numeral.)

Q5. A man presents to the clinic alarmed that his urine is reddish-brown in colour. He has haematocrit of 38% and MCH of 12.6 g/dL. His spleen is normal on examination. When his RBCs are mixed with antibodies specific for immunoglobulin, no agglutination occurs. Enter the code (I-X) corresponding to the type of anaemia which most likely accounts for these results. **(20 points)**
(Enter a roman numeral.)

Q6. A girl has presented to the clinic multiple times with fever and cough, with sputum cultures growing *Haemophilus influenzae*. She has also reported occasions of intense pain in her chest. Laboratory findings show haematocrit of 22%, haemoglobin of 6.5 g/dL, MCH of 30 pg/cell and MCV of 88 µm^3. Enter the code (I-X) corresponding to the type of anaemia which most likely accounts for these results. **(20 points)**
(Enter a roman numeral.)

Q7. A woman who gave birth to a healthy child two years ago has just delivered her second child. The newborn appears jaundiced and has an enlarged abdomen. It is previously known that the mother has blood type A, and the boy has blood type O. Enter the code (I-X) corresponding to the type of anaemia which most likely accounts for these results. **(20 points)**
(Enter a roman numeral.)

Q8. An elderly woman presents to the clinic complaining of weakness and malaise. She has experienced significant weight loss over the past 4 months. She also reports loss of appetite, nausea and vomiting, as well as having very dark-coloured stools in the recent few weeks. Blood test results reveal haematocrit of 33%, haemoglobin of 8 g/dL, MCH of 16 pg/cell, and MCV of 59 μm^3. A diagnosis of colorectal cancer is made. Enter the code (I-X) corresponding to the type of anaemia which most likely accounts for these results. **(20 points)**

(Enter a roman numeral.)

Answers and Explanations

This problem, while seeming to require content knowledge, can actually be solved through a careful analysis of the clues provided.

Q1.

Answer: **FTFT**

Explanation:

A. There is no obstruction of bile flow to cause post-hepatic jaundice where bilirubin made soluble by the liver cannot be excreted and thus accumulates as conjugated bilirubin; instead beta-thalassaemia causes pre-hepatic jaundice, as the haemolysis releases so much haem that bilirubin cannot be converted to its water-soluble form in time, resulting in unconjugated bilirubinaemia
B. New splice sites may be used only occasionally and hence only lead to a reduction in synthesis, whereas chain terminator mutations will lead to a complete absence of synthesis.
C. The preamble states that thalassaemias result from an imbalance in globin chain synthesis, so having deletions in both alpha and globin chains is likely to lessen the imbalance and result in milder forms of thalassaemia (beta-thalassaemia intermedia).
D. Individuals in population X are likely to inherit chromosomes that already possess two deletions, whereas those in Y are likely to inherit chromosomes that possess only one deletion.

Q2.

Answer: **VIII**

Explanation: MCV is high, which implies megaloblastic anaemia (large megaloblast cells which are RBC precursors due to ineffective erythropoiesis). Hence it can can only be pernicious anaemia or folate-deficiency anaemia. It cannot be iron-deficiency anaemia, as that will lead to microcytic anaemia i.e. smaller than normal RBCs. The gastric pH is higher than normal which could be due to achlorhydria. Further investigation reveals atrophy of the gastric mucosa, and urease breath test is used to test for *H. pylori* infection, which can cause atrophic gastritis.

Another cause of atrophic gastritis is the development of autoantibodies against parietal cells which secrete intrinsic factor. Since intrinsic factors are required for vitamin B12 absorption, this most likely results in the pernicious anaemia seen in the patient.

Q3.

Answer: **I**

Explanation: No agglutination in direct Coombs test rules out immunohaemolytic anaemia. From Table 1, red blood cells become spheroid instead of their usual biconcave shape, lowering their lower surface area to volume ratio, resulting in higher osmotic fragility, as seen in Figure 1.

Q4.

Answer: **II**

Explanation: From Table 1, anaemia is caused by haemolysis which occurs only during periods of oxidative stress. Since extravascular haemolysis by phagocytes in spleen is acute, not chronic, so splenomegaly is not observed. The bite cells are due to phagocytes removing inclusions from RBCs. Antimalarial drugs e.g. primaquine result in haemolytic anaemia in G6PD individuals due to increased oxidative stress. Individuals with G6PD cannot produce sufficient reduced glutathione to cope with the increased oxidative stress.

Since oxidative stress underlies G6PD deficiency, affected individuals are also resistant to malaria, explaining the higher frequency of G6PD deficient alleles in malaria-prone areas like Africa.

Q5.

Answer: **VI**

Explanation: From Table 1, PNH involves complement activity. There is intravascular, rather than extravascular haemolysis i.e. haemolysis does not occur within phagocytes, so free haemoglobin is oxidised to methaemoglobin which passes out in urine (reddish-brown). Intravascular haemolysis also accounts for normal spleen as phagocytes in spleen do not undergo hyperplasia.

Q6.

Answer: **III**

Explanation: Autosplenectomy results in a lack of splenic immune function, which increases susceptibility to infections by encapsulated organisms e.g. *Haemophilus influenzae*. The intense pain occurs due to pain crises caused by occlusion of blood vessels by sickle cells.

Q7.

Answer: **VII**

Explanation: Rh incompatibility occurs when initial pregnancy triggers development of anti-Rh antibodies, which causes erythroblastosis faetalis in second child.

Q8.

Answer: **X**

Explanation: Excessive bleeding from the tumour has resulted in iron deficiency.