Prelims Round - Subjective Section

August 2022

Instructions

- The duration of the exam is 4 hours which is **inclusive** of any unforeseen delays in scanning and uploading. The **hard** deadline is set at **10:00 PM IST, 20th July 2022**. Any submission after that will not be considered. Only one submission per team will be allowed.
- We highly encourage you to start the process of submitting your answers in the Google form (see below) by 10:00 IST. Late submissions, in any case, will not be accepted. If you face persisting network issues during submission, inform us immediately by email (**bioblitz.pravega@gmail.com**) or call Prithu at **+91 7595811875** or Kredai at **+91 9840700957**.
- This paper contains **6 subjective questions** in total and a total of **60 marks**
- Mention question number and title (which is given at the beginning of each question) clearly. We advise writing the question number at the middle of the page to avoid it being cut out during scanning. Answer all subparts of a question together and begin each question in new page. Clearly mention the subpart number for each question.
- Clearly write your name(s), the name of your institute, as well as your registered email ids at the beginning of the answer script.
- Do not submit multiple documents. If you have both typed and handwritten answers, compile your documents as a **single** pdf or word file.
- Show your steps clearly for both short answer questions and long answer questions. Do not skip steps to receive full credit. Partial credit may be awarded for an incomplete solution or progress towards a solution.
- In case of any clarification required kindly mail us or ask us on the discord server established. We will try our best to clarify. Even after that, if you feel you have any comments regarding the question (for example, incompleteness, incorrectness, etc.) you can mention that in your answer clearly with all the reasons why you think so. If we feel that you claim is correct, scoring will be done accordingly. But the first priority should be getting it clarified from us.

Questions

1 (*15 Marks*) Transposons

Barbara McClintok is considered to be one of the most influential women in science, having won the Nobel Prize in 1948 for her discovery of transposons. Here, we'll try to trace how she discovered them.

1.1 The Maize

History

In the 1930's, Barbara was working with X-ray induced maize and it was here that she saw some interesting results

Background

The maize kernel properties are derived from 4 genes, all on Chromosomes 9.

1.1.1

What is the ploidy of maize kernels (**Hint:** They are derived from the aleuron layer of maize endosperm)

- n
- 2n
- 3n
- 4n

The 4 genes are as follows:

C gene: It has 3 alleles:

1. C: This causes blue pigmentation in the kernels.
2. c: This does not cause blue pigmentation.
3. C^i: This inhibits the pigment causing machinery.

Order of dominance: $(C^i)>(C)>(c)$

Bz gene: It has 2 alleles:

1. Bz: This is the normal allele.

2. bz: This is the recessive mutant which converts blue pigmentation to bronze pigmentation.

Order of dominance: $Bz > bz$

Wx(waxy) gene: It has 2 alleles:

1. Wx: This is the normal allele
2. wx: This is the recessive mutant which converts normal starch to waxy starch.

Order of dominance: $Wx > wx$

Sh(shrunken) gene: It has 2 alleles:

1. Sh: This is the normal allele
2. sh: This is the recessive mutant which gives shriveled rather than plump kernels.

Order of dominance: $Sh > sh$

1.1.2

Draw a Punnett square of cross between a having genotype: $CCbzbzwxwxshsh$ and having genotype $C^iC^iBzBzWxWxShSh$ (consider 0% recombination frequency for all genes involved.)

1.2 The experiment

The Ds Locus

Coming on to actual experiment: McClintok conducted a cross between the X-ray induced () with (CC). She noticed that along with the majority of clear kernels, some of the kernels had blue patches.

What she deduced from this this was that during development, some of the cells underwent a partial deletion of the part containing gene on the chromosomes from parent, which manifests itself in the blue patches. On conducting similar cross with having CCshshbzbzwxwx and having ShShBzBzWxWx, she found something similar. Instead of usual phenotype, which you found in question 2, she found that the kernels were clear with bronze patches. Moreover, all bronze patches were waxy and shrunken. What this meant was that chromosomes were getting broken from. the same locus. As probability that different cells in the kernels underwent mutation at same place this many times is very less, McClintok deduced that there was a locus on Chromosome 9, which was responsible for this breakage. She confirmed this cytologically by seeing the chromosome under microscope, which showed that the chromosomes broke at the same place always.

Figure 1: Different corn kernels as seen by Barbara

1.2.1

Consider that there is a gene Ac (activator), which causes breakage at Ds, and which may not necessarily be on the chromosome 9. Also consider that the pollen plant is heterozygous for this allele. Predict what and in what proportions will the progeny be when back-crossed.

Indeed, Barbara found that Ac was in-fact on different chromosome than 9. When she tried to map Ac, she was surprised to find that Ac mapped to different regions of chromosomes in different cells. Did Ac move in the genome?

Next Observation

Another observation surprised McClintok. In one of the derivatives of the 'special stock' she had been studying, she found out that the progeny were 'clear-kernels and blue-patched' instead of 'brown-patched', and the patches were plumped and non-waxy.

1.2.2

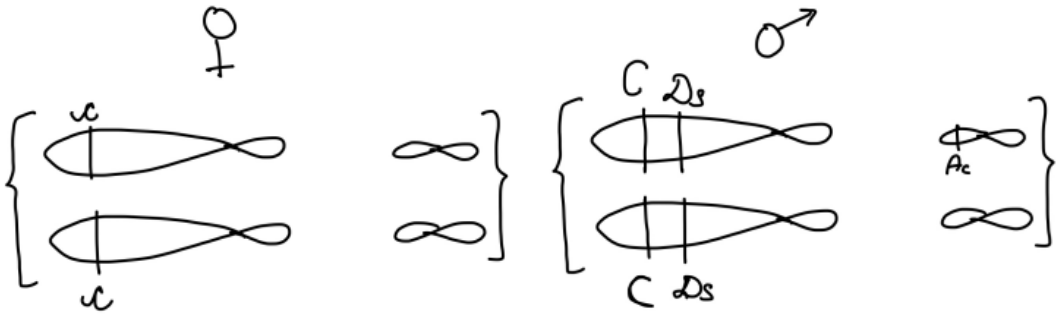
Choose a suitable reason for this observation. Give reason (in very brief) as to why others are not valid:

- Ds mutated and was rendered inactive
- Other genes were restoring the phenotype
- Ds moved in between Sh and C

- Due to crossing over

1.3 The last piece

The last piece of evidence, that convinced Barbara that genes were in-fact moving, was as follows: Consider a cross as follows:



1.3.1

Predict the phenotype and their respective frequencies in progeny. Barbara did observe the expected frequencies and phenotype, but in very rare occurrences, she found out that the phenotype was 'reversed', i.e., some kernels had 'blue-patches' in clear kernels. Later studies revealed that Ds had moved to inside C gene, rendering it inactive (effectively creating a new allele c-m-1). This can be confirmed by cytological experiments. What happened was that in presence of Ac, Ds again 'jumped' out of C in some cells, causing blue patches.

In the end, or is it?

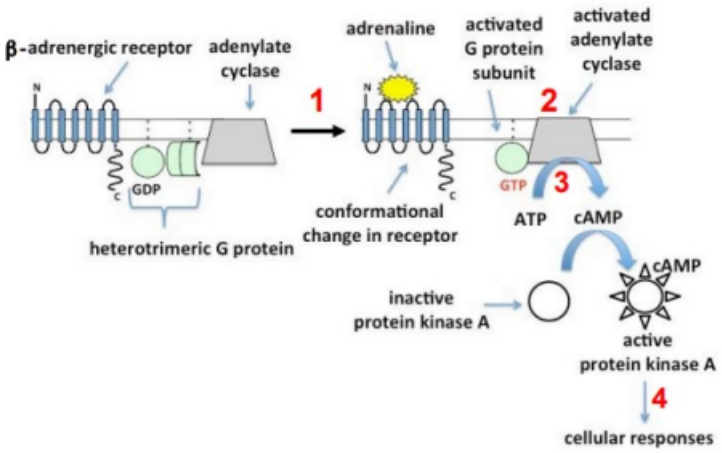
These three observations made Barbara to forego the static genome idea, instead she started searching for such 'jumping' genes, which excised from one place and 'transposed' to other. And this was just the beginning.....

Reference

Refer to 'Introduction to Genetic Analysis' by A.J.F. Griffiths and William Gelbart

2 (*13 Marks*) Contacting Cells

The diagram below shows a signaling pathway that is activated following the binding of adrenaline to the β-adrenergic receptor. The major steps of this signaling pathway are shown on the diagram.



The cAMP produced, diffuses through the cell to bind and activates Protein kinase A (PKA). PKA affects many proteins in the cell by attaching a phosphate group to them. The phosphorylated proteins induce cellular changes that mediate the increased heart rate and accounts for the flight-or-fight response.

2.1

Why is the effect of adrenaline limited to a specific cell type in an individual?

2.2

Identify the first step in the signaling pathway where the signal starts to get amplified: Step 1/ Step 2/ Step 3/ Step 4

2.3

Consider the following homozygous mutations in different components of this pathway.
M1: The β-adrenergic receptor constitutively stays in its ligand bound form
M2: Adenylate cyclase can no longer bind to ATP
M3: PKA continues to stay in its active form
M4: The cell produces a variant of β-adrenergic receptor that lacks 4 of its 7 transmembrane domains Complete the table for the cells that are incubated with

adrenaline and have the following mutations. Predict the change in heart rate by comparing the mutations with control, wild type, adrenaline treated cells.

Mutants	cAMP concentration (*High/ Low*)?	PKA activated (*Yes/ No*)?	Does the heart rate *increase OR decrease in the presence of adrenaline*?
M1 & M3			
M2 & M4			
M2 & M3			

3 (*10 Marks*) DNA Topology

In a DNA molecule, various topological domains exist. In a free linear DNA due to the free end rotation, it is easy for the DNA to unwind. In case of an actual DNA molecule, it is either existing, attached to the nuclear matrix, attached to the nuclear memebrane or attached in between two binding protein molecules making it difficult for them to unwind

There is another topological domain that we observe, circular DNA where it is bound on every end via covalent bonds making it difficult to unwind, thus supercoiling occurs. We have one term known as Linking number which is topologically invariant.

Linking number = L_k

The other two terms that we have are Writhe and Twist. Writhe refers to how many times a single DNA strand on looping around, cuts itself and is essentially 0 for a closed circular loop. Linking number is the number of times one DNA piece cuts the other if the other is considered as an open loop. Twist is essentially the same as linking number when writhe is 0.

$L_k = T_w + W_r$

When a piece of DNA supercoils it is negatively supercoiled if it unwinds and positively supercoiled when it overwinds. Negative supercoiling is most well studied and in general is energetically unfavourable.Negative supercoiling of solenoidal topology is well observed in eukaryotes. Supercoiling results in a change in Linking number and τ is a term that takes it into consideration.

N be the number of base pairs and number of base pairs per DNA turn is γ.

τ = L_k - L_{k0} (L_{k0} is the open circular loop linking number)

Supercoiling density is $\sigma = \tau.\gamma/N$ The free energy of a negatively supercoiled DNA molecule can be approximated as $\Delta G = 10RT\sigma^2$ for our standard Watson Crick DNA. Approximation based on the difference in elastic energies stored in the two configurations.

3.1

Many a times in order to overcome the torsional stress developed due to supercoiling , the DNA locally unwinds in certain regions. For example it has been observed that in a certain bacterial species with 1050 nucleotides, the torsional strain due to supercoiling is balanced by the unwinding of 42 base pairs. In case the bacteria had

2100 nucleotides, unwinding of how many base pairs would be required.(One can approximate the energy to break A-T and G-C to be very close to each other)

3.2

In some Eukaryotes with 1050 nucleotides, it was observed that rather than 42, opening up of 21 base pairs were only seen to stablise the DNA. Come up with a hypothesis to explain this observation. (Hint :- Look towards what else could lead to stablisation of a DNA molecule)

3.3

Do you expect the supercoiling in Z-DNA to be favourable or unfavourable. Explain your choice.

3.4

In case we replace B-DNA by Z-DNA then the energy formula changes. What do you think would be the new formula for ΔG now. (There is change in interaction energies in B-DNA and Z-DNA. λ is defined as the ratio of total interaction energy in Z-DNA and in B-DNA. You can also use any other constants that you want.

3.5

Aarti is a lab intern who wants to perform a DNA gel electrophoresis. She aldready ran it for a E.Coli culture at 25^oC and got her results. However, she wanted more DNA molecule as her cell culture was small, so she kept her culture in the incubator. However, she incorrectly kept the temperature as 38^oC rather than 30^oC. Now when she will run the gel electrophoresis what do you think she will see.

3.6

In the earlier problem assume that the incubator has a power of 40W and she ran it for 2 hours. Take that 2 perecnt of the total heat given by the incubator is used to change the temperature of the DNA molecules in the cell. Neglect the heat capacity of cells and DNA in this case. What would be the change in linking number. DNA is B-DNA and E.Coli has 4.6 million nucleotides.

4 (*6 Marks*) Santa Claus

Without loss of generality we may assume that Santa Claus, apart from being a philanthropist, is also a genetic engineer.

As a test for elf lab assistants, he quizzed the applicants one midnight noon -

" Consider an environment where only two of the usual four nucleotides are available - C (because it's my initial) and G (for obvious reasons)."

"Typical functional proteins are rarely smaller than 50 amino acids long. How many distinct 50-amino-acid polyptides can be made in our imagined environment? "

"A particular interior portion of an mRNA sequence was isolated, which reads - 5'...CC*CGCGG*CC*CGGGGCCC*GC*GGCCCCCG*GGGCC*GC*GG*CC*CGG GGGCCCGCGGGGCCCGCC...3' The blanks signify bases that couldn't be determined experimentally - can they be found otherwise?"

One of the elves didn't take the time to copy down the whole sequence, for he already realised which well-known protein it was related to. What was the elf thinking of?

(All references are allowed.)

(The asterisk are the gaps that need to be filled)

(Assumptions: cellular machinery is intact except for the given condition. Only initiation and stop codons differ from real life.)

5 (*10 Marks*) The Gordian Knot

Despite being single-stranded, RNA molecules often exhibit a great deal of double-helical character. This is because RNA chains frequently fold back on themselves to form base-paired segments between short stretches of complementary sequences. Base pairing can also take place between sequences that are not contiguous to form complex structures aptly named pseudoknots. Also, RNA can foldup into complex tertiary structures frequently involving unconventional base pairing (non-Watson-crick base pairing), such as the base triples and base–backbone interactions seen in tRNAs.

Murine leukemia virus (MLV) is an RNA virus that causes cancer in mice and certain other vertebrates. Like the human immunodeficiency virus (HIV), it is a member of a class of RNA viruses known as retroviruses and has mRNA as its genome. The mRNA codes for a structural protein, Gag, and a polyprotein composed of Gag and a reverse transcriptase enzyme, Pol. The differential amount of translation of the structural protein and the enzyme is controlled by a pseudoknot in the mRNA which lies between the *gag* and the *pol* genes and allows the ribosome to bypass the stop codon in between if both *gag* and *pol* (for producing the enzyme) is required.

The pseudoknot exists in two forms: "Active form" which allows the ribosome to bypass the stop codon, and "Inactive form", which stops translation at the stop codon. The pseudoknot acts as a proton sensor. At physiological pH, the concentration of protons is such that only 5%–10% of the pseudoknots sense the protons and fold into the active conformation. To achieve this, the molecule uses the N1 nitrogen atom of adenine, which is not generally protonated, to acquire a proton and form a triple base in the molecule and change its conformation to the active form.

5.1

Murine Leukemia Virus has the following as its genome:

- +ssRNA
- -ssRNA
- +ssDNA
- dsDNA

5.2

It is known that the base triple formed in the active form of the pseudoknot is a U:A:U triple.

Draw the structure of the base triple, clearly showing the hydrogen bonds between the three bases. You have to only draw the N-bases. No need to draw the ribose sugars.

If it is known that the structural protein Gag is required much more than the enzyme, which of the genes, *gag* or *pol*, should be upstream, before the pseudoknot (in the 5'→3' direction).

5.3

Draw an appropriate graph of the amount of DNA that codes for the Gag protein, and pH.

6 (*6 Marks*) Down the Helix

The value of 10 bp per turn of B-DNA varies somewhat under different conditions.

A classic experiment that was performed in the 1970s showed that DNA absorbed on a surface has somewhat greater than 10 bp per turn.

Short segments of DNA were allowed to bind to a mica surface. The presence of 5'-terminal phosphates on the DNAs held them in a fixed orientation on the mica.

The mica-bound DNAs were then exposed to DNase I, a deoxyribonuclease that cleaves the phosphodiester bonds in the DNA backbone. Because the enzyme is bulky, it is able to cleave phosphodiester bonds only on the DNA surface furthest from the mica (think of the DNA as a cylinder lying down on a flat surface) because of the steric difficulty of reaching the sides or bottom surface of the DNA.

As a result, the length of the resulting fragments should reflect the periodicity of the DNA and the number of base pairs per turn. Now, the cleaved pieces of DNA are subjected to gel-electrophoresis, where bands of different lengths of DNA get separated.

6.1

If the average number of nucleotides per turn of DNA is 10.5, then bands of the following lengths will be obtained (more than 1 may be correct, NO partial marking):

- 21
- 25
- 15
- 42

6.2

Would this method be helpful if the number of base pairs per turn in DNA would have varied? Explain why or why not.