Multiple Choice Questions

This section tests how well you understand and remember the concepts covered during the lesson, with a bit of application of knowledge required. For each question, select one option (or more, if applicable).

1. In Sanger sequencing, why do ddNTPs terminate DNA synthesis?
   A. They lack a 5’ phosphate group necessary for elongation.
   B. They lack a 3’ hydroxyl group necessary for elongation.
   C. They are degraded by DNA polymerase.
   D. They cannot be recognised by DNA polymerase.

2. ddNTPs are an essential component of Sanger sequencing. However, their concentrations may determine the effectiveness of the reactions. What is the ideal ddNTP concentration?
   A. Equal to dNTP concentration.
   B. Much lower than dNTP concentration.
   C. Much higher than dNTP concentration.
   D. It does not matter.

3. Which of the following factors most directly limits the maximum read length in Sanger sequencing?
   A. The resolution of the capillary electrophoresis system.
   B. The efficiency of ddNTP incorporation.
   C. The purity of the DNA to be sequenced.
   D. The fidelity of the DNA polymerase used.

4. What is the primary reason that only one primer used in Sanger sequencing?
   A. To prevent exponential amplification of the target sequence.
   B. To ensure only one strand of the DNA template is sequenced at a time.
   C. To improve the accuracy of the sequencing reaction.
   D. To save resources.

5. Which of the following steps is unique to Maxam-Gilbert sequencing compared to Sanger sequencing?
   A. Use of chain-terminating dideoxynucleotides.
   B. Generation of DNA fragments of different sizes.
   C. Fluorescent labelling of DNA molecules.
   D. Chemical cleavage of DNA at specific bases.

6. What is a key difference between Sanger sequencing and next-generation sequencing (NGS)?

A. Sanger sequencing uses fluorescent labels, while NGS does not.
B. NGS sequences DNA in parallel, while Sanger sequencing is a serial process.
C. NGS uses chain-termination technology, while Sanger sequencing does not.
D. Sanger sequencing can be used to sequence RNA.

7. A researcher is studying a newly discovered bacterial strain and wants to:

1. Sequence the entire genome (approx. 5Mbp)
2. Identify epigenetic modifications in the genome
3. Detect low-frequency mutations in cell populations across the genome

The table below displays some sequencing technologies the researcher is considering.

Technology	Key Features	Strengths	Limitations
Sanger Sequencing	Small-scale sequencing via chain-termination PCR	Highly accurate for short regions	Low throughput
Illumina Sequencing	Short-read, high-throughput sequencing	Highly accurate reads and cost-efficient	Shorter reads
Nanopore Sequencing	Long-read sequencing with electrical signals	Very long reads	High error rates
PacBio Sequencing	Long-read sequencing with native modification detection	Highly accurate reads	Lower throughput, higher cost

Based on the information above, which combination of technologies would be most effective in achieving all three objectives efficiently?

A. Illumina sequencing for (1) and (2) and Sanger sequencing for (3).
B. Illumina sequencing for (1) and Nanopore sequencing for (2) and (3).
C. PacBio sequencing for (1) and (2) and Illumina sequencing for (3).
D. PacBio sequencing for (1) and (2) and Nanopore sequencing for (3).

8. A researcher sequenced a DNA region of interest (870 nucleotides) from a patient and identified the following mutations:

1. **c.78C>G:** A missense mutation at the 78th nucleotide (C→G) that changes the catalytic histidine residue to a glutamine.
2. **c.150ins:** A dinucleotide insertion at the 150th nucleotide.

3. **c.312A>C:** A synonymous mutation occurring in the last base of an exon.

Which of the following mutation(s) are most likely to affect protein function?

A. **c.78C>G**
B. **c.150ins** and **c.312A>C**
C. **c.78C>G** and **c.312A>C**
D. **c.78C>G** and **c.150ins**

True-False Statements

This section comprises questions in a common format tested in IBO papers. It requires application of knowledge to solve the questions. For each statement, mark each either True or False.

1. A bioinformatics team is tasked with assembling the genome of a novel bacterium from a deep-sea hydrothermal vent. Key considerations include:

a. Resolving repetitive regions of the genome
b. Correcting sequencing errors caused by specific technologies
c. Detecting possible epigenetic modifications like methylation

The table below displays some sequencing technologies the researcher is considering.

Technology	Key Features	Strengths	Limitations
Sanger Sequencing	Small-scale sequencing via chain-termination PCR	Highly accurate for short regions	Low throughput
Illumina Sequencing	Short-read, high-throughput sequencing	Highly accurate reads and cost-efficient	Shorter reads
Nanopore Sequencing	Long-read sequencing with electrical signals	Very long reads and detects modification	High error rates
PacBio Sequencing	Long-read sequencing with native modification detection	Highly accurate reads	Lower throughput, higher cost

Based on the information above, mark each statement either True or False.

A. Illumina sequencing is suitable for generating short reads with low error rates, but it may struggle with repetitive regions.
B. PacBio sequencing generates longer reads, which can resolve repetitive regions but typically has higher raw error rates than Illumina.reg
C. Methylation patterns can be directly detected using Illumina sequencing if bisulfite conversion is performed.
D. Combining Illumina and Oxford Nanopore sequencing can balance error correction, long-read assembly, and epigenetic analysis.

2. A researcher is studying the effects of mutations in the p53 tumor suppressor gene. His goals are:

a. Identifying mutations in conserved domains
b. Predicting how amino acid substitutions affect protein function
c. Designing primers to amplify exons of interest for further analysis

Mark each statement either True or False.

A. Mutations in conserved domains of p53 are more likely to disrupt its function than mutations in non-conserved regions.
B. Substituting glycine with alanine in the p53 protein core is likely to destabilize the structure.
C. Primer design for amplifying p53 exons should include sequences complementary to intronic regions flanking the target exon.
D. Silent mutations in the p53 coding region cannot affect its tumor suppressor activity.

3. A population geneticist is studying genetic diversity in an endangered species using RFLP. Their pipeline involves:

a. Identifying polymorphic restriction sites in the genome
b. Comparing DNA fragment patterns across individuals
c. Using the results to infer population structure

Mark each statement either True or False.

A. Polymorphic restriction sites are necessary for RFLP analysis to distinguish between individuals in a population.
B. DNA fragments with similar sizes can be misinterpreted as identical in RFLP if electrophoresis resolution is not powerful enough.
C. RFLP is a suitable method for detecting large-scale structural variants in genomes.
D. The loss of a restriction site due to a point mutation can be detected as a difference in fragment length during electrophoresis.

4. A molecular biologist is mapping restriction sites in a plasmid to confirm successful insertion of a gene of interest. The steps include:

a. Digesting the plasmid with single and double restriction enzyme digests
b. Analysing the fragment pattern via gel electrophoresis

c. Comparing experimental results to theoretical predictions

Mark each statement either True or False.

A. Discrepancies between observed and predicted fragment sizes could indicate the presence of mutations or unexpected insertions.
B. Double digestion with two restriction enzymes is always more informative than single digestion.
C. Restriction mapping can determine the orientation of an inserted gene relative to the plasmid backbone.
D. A successful ligation reaction guarantees that the gene of interest has been inserted into the correct site in the plasmid.

5. A forensic analyst is comparing DNA from a crime scene with that of two suspects using DNA fingerprinting. The analysis involves:

a. Amplifying short tandem repeats (STRs)
b. Performing capillary electrophoresis to determine STR lengths
c. Calculating the statistical probability of a random match

Mark each statement either True or False.

A. A suspect with identical STR profiles to the crime scene sample is definitively guilty.
B. STR profiles can vary between monozygotic twins.
C. A DNA sample degraded by environmental exposure can cause incomplete STR profiles.
D. The probability of a random match increases as more STR loci are analysed.

6. Two individuals in a lab had to use different techniques to quantitatively analyse their samples of DNA. The virologist is studying the replication dynamics of a novel virus using qPCR. They aim to:

a. Quantify viral RNA levels in infected cells over time
b. Compare the efficiency of two antiviral drugs based on their effects on viral replication

Mark each statement either True or False.

A. qPCR reactions can proceed using viral RNA as a template.

B. The amplification efficiency of qPCR needs to be close to 100% for accurate quantitation.
C. Comparing Ct values between treatments allows estimation of absolute changes in viral RNA levels.
D. SYBR Green binds to single-stranded DNA formed in qPCR.

The biochemist is optimising DNA extraction from plant tissues for sequencing. Their considerations include:

a. Removing proteins to ensure high DNA purity.
b. Quantifying DNA using spectrophotometry.

Mark each statement either True or False.

A. A260/A280 ratios above 1.8 indicate contamination with proteins.
B. Phenol contamination increases the A260/A280 ratio above the normal range.
C. Pure DNA typically shows an A260/A280 ratio of 2.0, indicating optimal purity.
D. Presence of salts may decrease A260/A230 ratio.

IBO Questions

These questions are sourced directly from the International Biology Olympiad (IBO) and require critical thinking as well as sound application of knowledge.

Question 1.

Q9

Bulb1 and Bulb2 are transcription factors involved in the survival response of plants exposed to UV radiation. Wild-type (wt) and homozygous mutant (*Bulb1-/-*) *Arabidopsis* plants were exposed to UV-B for various times and their RNA extracted. RNA was dissolved in 20 µl of buffer. A 2 µl aliquot was taken from one of the stock solutions and diluted to 1 ml. The absorbance of the solution at 260 nm was found to be 0.046.

Q.9.1 Given that a 40 µg/ml solution of RNA gives an absorbance of 1.0 at 260 nm, calculate the concentration of RNA in the stock solution in µg/ml.

Q.9.2 In each experiment the scientists carried out, they are required to use 2 µg of RNA. What volume of stock would they need to use? Give your answer in µl, rounded to two decimal places.

Q.9.3 How many experiments could the researchers conceivably carry out with the original stock of RNA?

The RNA samples were used for measurement of *bulb2* transcript levels by quantitative PCR (Figure 1A). In a different experiment Bulb1 and Bulb2 protein levels in wild-type *Arabidopsis*, exposed to UV-B radiation for various times were assessed (Figure 1B).

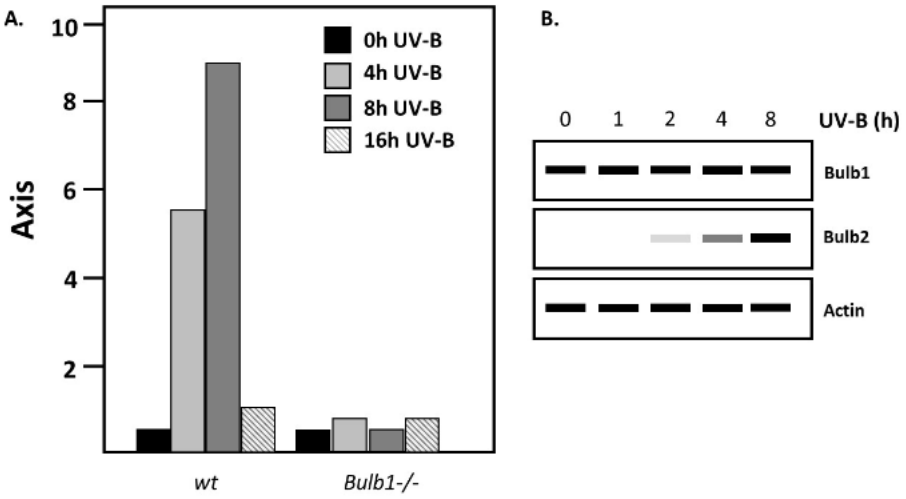
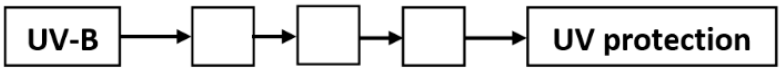


Fig.1. Axis = Fold change relative to control. *wt* = Wild-type

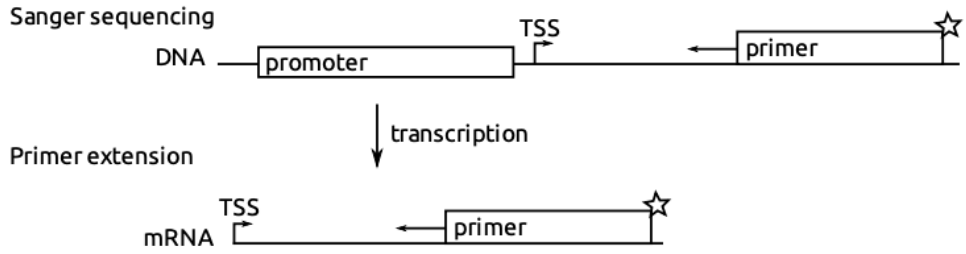
Q.9.4 Build a model of UV-B response in *Arabidopsis* based on the available information.

A. *Bulb1* transcription
B. *Bulb2* transcription
C. Bulb1 translation
D. Bulb2 translation
E. Bulb1 degradation
F. Bulb2 degradation
G. Bulb1 nuclear translocation
H. Bulb2 nuclear translocation

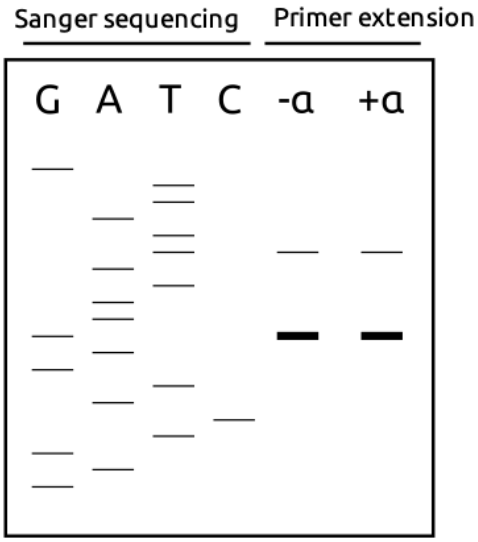


Question 2.

To determine the precise transcription start site (TSS) of a newly discovered bacterial gene promoter, a radioactively labeled primer complementary to the 3'-end of the gene is used both for Sanger sequencing of the DNA construct and for primer extension of the mRNA. Primer extension (similar to cDNA synthesis) is repeated on mRNA transcribed with addition of the transcription factor α.



The fragments obtained are separated by gel electrophoresis, a radiography is presented below.



Indicate if each of the following statements is true or false.

A. Different polymerases are used for the Sanger sequencing and primer extension assays.

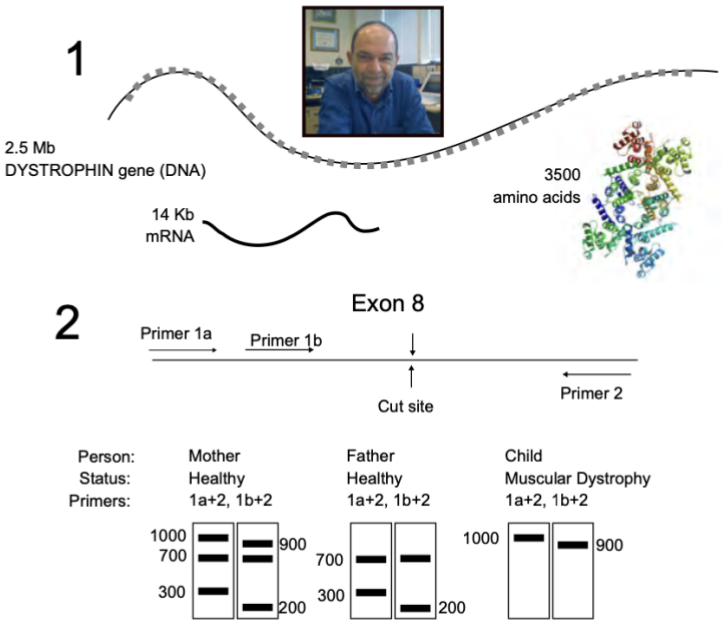
B. mRNAs of this gene with CUCAUGAC as the first eight bases after the TSS are found in these cells.

C. Multiple TSS exist for this gene.

D. Transcription is modulated by transcription factor α.

Question 3.

Muscular Dystrophy is caused by alterations to *DYSTROPHIN* (1). This is a very large gene, and its length affects the biology and diagnosis of Muscular Dystrophy.
RNA polymerase moves along DNA at 30 bp per second.
DNA polymerase has an error rate of 10^{-8} mistakes per base. Repair systems later correct 99% of mistakes.
Sir Alec Jeffreys (1950-present) invented DNA fingerprinting (RFLPs) whereby *DYSTROPHIN* exons may be amplified by PCR, treated with DNA-cutting enzymes (endonucleases), and separated on an agarose gel, according to length in bp (2).



A. Calculate the time taken for RNA polymerase to transcribe DYSTROPHIN.

	1 second	1 minute	1 hour	10 hours	1 day
Select the nearest time to the correct answer.					

B. Calculate the number of approximate number of errors made during replication of DYSTROPHIN.

	50	500	5000	50000	5000000
Choose the nearest number to the correct answer.					

C. Deduce the approximate distance of each site from the start of Exon 8.

	100 bp	200 bp	300 bp	700 bp	1000 bp
Primer 1b binding site.					
Endonuclease cut site					
Primer 2 binding site.					

D. Mark each statement True or False.

	True	False
DYSTROPHIN is on the X chromosome.		
Muscular Dystrophy is dominant.		
DYSTROPHIN protein could be made in bacteria with a 20 kb plasmid.		
Many muscular dystrophy patients have new (*de novo*) causal mutations.		

Question 4.

You find a mutation in p53 ('the guardian of the genome') at nucleotide 42. In your sequence, it is an adenine (A), whereas it is usually guanine (G) in the wild-type, as shown in Figure 1.

Fig.1

Q.7.1 You design a test for the mutant allele using restriction enzymes. Indicate with an **X** which enzyme (A-D) can be used to distinguish patient samples?
A. MboII
B. BglII
C. DpnI
D. HgaI

A clinician uses the appropriate enzyme to carry out restriction fragment length polymorphism analysis. Results for patient A-G are shown in Figure 2.

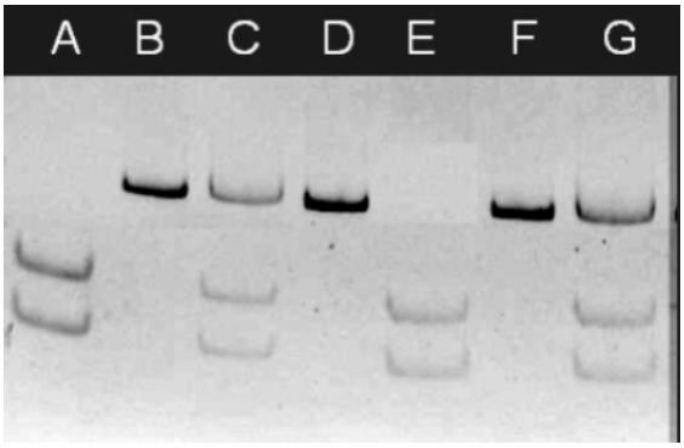


Fig.2

Q.7.2 Determine the genotype of the patients by marking in it in the table with an **X**. The letters in the table refer to the nucleotides found at position 42.

Q7.2	A	B	C	D	E	F	G
GG							
AG							
AA							

Question 5.

The Table below shows the genetic codes of amino acids.

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Some viruses (e.g. tobacco mosaic virus (TMV)) have RNA sequences that contain a "leaky" stop codon. In TMV 95% of the time the host ribosome will terminate the synthesis of the polypeptide at this codon but the rest of the time it continues past it.

The following sequences show part of a mRNA from TMV. Indicate the sequence(s) that may result in two polypeptides in the indicated frame with a tick (✓) and those that will not with a cross (✗). (1.8 points)

a. 5′-AUG-UCU-UGU-CUU-UUC-ACC-CGG-GGG-UAG-UAU-UAC-CAU-GAU-GGU-UAA-3′

b. 5′-AUG-ACC-CGG-GGG-UUU-CUU-UUC-UAG-UAU-GAU-CAU-GAA-GGU-UGU-UAA-3′

c. 5′-AUG-CUU-UUC-UCU-UAU-UAG-CAU-GAU-GGU-UGU-ACC-CGG-GGG-CCC-UAA-3′

d. 5′-AUG-CAU-GUU-CUU-UUC-UCU-UAU-UGU-GGU-UGU-ACC-CGG-GGG-UUC-UAA-3′

e. 5′-AUG-CAU-GAU-GGU-UGU-ACC-CGG-GGG-UAG-CUU-UUC-UCU-UAU-UGC-UAA-3′

f. 5′-AUG-UCU-UAU-UGG-CAU-GAU-GGU-UGU-CUU-UUC-ACC-CGG-GGG-AAA-UAA-3′

Question 6.

Arrange the order of the DNA molecules from lowest to highest in terms of their melting temperature (Tm). (0.9 points)

a. 5′-AAGTTCTCTGAA-3′

   3′-TTCAAGAGACTT-5′

b. 5′-AGTCGTCAATGCGG-3′

   3′-TCAGCAGTTACGCC-5′

c. 5′-GGACCTCTCAGG-3′

   3′-CCTGGAGAGTCC-5′

Question 7.

Research was conducted to examine the presence of regulator element in the upstream of transcription start site from eukaryotic gene. As a preliminary study, a researcher performed *in silico* analysis by multiple alignment of nucleotide -37 to -26 from 900 different genes. The resulting homology percentage data are shown in the table below.

		-37 (5')											**-26 (3')**
Base frequency (%)	A	21	16	4	91	0	95	67	97	52	41	16	24
	C	23	39	10	0	0	0	0	0	0	9	35	37
	G	28	35	3	0	0	0	0	3	12	40	38	30
	T	28	10	83	9	100	5	33	0	36	10	11	9

4.1. Based on the given data, predict the most likely nucleotide sequence -35 to -29 within the conserved area which is essential for its regulator function. **In the Answer Sheet**, fill the boxes with A, C, T, and G, at the appropriate positions. (1.4 points)

	-35	-34	-33	-32	-31	-30	-29	
5'								3'

4.2. Deletion of nucleotides -50 to -26 of several genes resulted in dramatically decreased RNA polymerase binding within the gene. Which type(s) of sequence element may be represented by nucleotides -50 to -26? Indicate appropriate answer(s) with a tick (✓) and inappropriate answer(s) with a cross (×) **in the Answer Sheet**. (1.0 point)

Sequence element				
Operator	Promoter	Origin of replication (ORI)	Telomere	Enhancer

Question 8.

You are studying a small protein-coding locus the sequence of which is shown below. The sequence is shown from the 5' transcriptional START position to the 3' transcriptional STOP position. This part is responsible for coding of two different polypeptides. There is a short intron sequence which is shown with **BOLD** letters here.

```
          5.        10.       15.       20.       25.       30.       35.       40.       45.       50.       55.
5´ –CTACGTACTATGTATTCC**GATCTATA**CTCGATCTAGTCGCATTCCGATAAGATCGTAC– 3`
3´ –GATGCATGATACATAAGG**CTAGATAT**GAGCTAGATCAGCGTAAGGCTATTCTAGCATG– 5`
```

Q.6.1 How many nucleotides long would the final processed mRNA made from this gene be (not including the 5' cap and the 3' polyA tail)?

Q.6.2 Indicate with an **X** which strand is used as a template in transcription for the shorter polypeptide, the 5'-3' strand or the 3'-5' strand (read in a left → right direction)?

Q.6.3 What is the ratio of the numbers of amino acids in longer/shorter polypeptide (including the amino acid built in because of the START codon)? Give your answer to the nearest second decimal place!

Q.6.4 What is the third amino acid of the shorter polypeptide, if the Met is counted to be the first one? Add the one-letter code of amino acid based on the **codon table** in the beginning of your question sheet!

Q.6.5 Say that the C:G base pair that is underlined in the sequence above were changed to an A:T basepair to make a mutant form of this gene. Indicate with an **X** which kind of mutation would this be for the shorter polypeptide?
A. a frameshift mutation
B. a nonsense mutation
C. a silent mutation
D. a missense mutation

Question 9.

In recent years, a genome editing technology called the CRISPR-Cas9 method has been widely used for biology research. In the CRISPR-Cas9 method, an enzyme called Cas9 is guided to the target gene by forming a complex with a guide RNA with a sequence complementary to a part of the target gene. Then, Cas9 cleaves the double-stranded DNA of the target gene specifically with its activity of cleaving double-stranded DNA. Cas9 recognizes a 3-base sequence (NGG) called PAM sequence and cuts the DNA strand 3 to 4 bases upstream of PAM. The cleaved DNA chain is repaired by the DNA repair system, but at that time, a few bases are frequently deleted or inserted.

The CRISPR-Cas9 method was applied by targeting the region close to the translation start codon of the most upstream exon of a gene encoding enzyme A of a certain animal. The base sequence of the target region was determined for each of the four mutants obtained (Figure 1).

Original sequence	TA TCT TAC ATG ATC CTA CAA GTA CCT TAC GCT CGG CAG GAA G
Mutant 1	TAT CTT ACA TGA TCC TAC AAG TAC CTT ACA GCT CGG CAG GAA G
Mutant 2	TAT CTT ACA TGA TCC TAC AAG TAC CTT GCT CGG CAG GAA G
Mutant 3	TA TCT TAC ATG ATC CTA CAA GTA CCT GCT CGG CAG GAA G
Mutant 4	TA TCT TAC ATG ATC CTA CAA GTA CCT TAA CTC GCT CGG CAG GAA G

[ ]: Pam sequence recognized by Cas9

Start codon : ATG (underlined)

Stop codon : TAA, TAG, TGA

Figure 1

Indicate whether each of the following statements is true or false.

A. It is highly likely that the activity of enzyme A is retained in mutant 1. 64

B. It is highly likely that the activity of enzyme A is retained in mutant 2. 65

C. It is possible that the activity of enzyme A is retained in mutant 3. 66

D. It is highly likely that the activity of enzyme A is lost in mutant 4. 67

Answers

Multiple Choice Questions

1. **B**. DNA polymerase extends in the 5'→3' direction, and without the 3' OH group on the ddNTP, DNA polymerase cannot add an oncoming dNTP.
2. **B**. If the ddNTP:dNTP concentration was 1:1 or higher, DNA fragments generated would terminate very early on and there would be little chance for the entire DNA template to be sequenced. Conversely, a low ddNTP concentration ensures that ddNTPs are incorporated relatively infrequently and fragments of various lengths can be generated.
3. **A**. As DNA fragments increase in size, the difference in migration rates becomes increasingly small (take for instance a 1000bp fragment vs a 1001bp fragment – they are impossible to distinguish on a normal gel). The other options may affect maximum read length, but not in such a drastic manner.
4. **B**. This allows DNA sequencing data to be produced in a defined direction. If two (forward and reverse) primers were used like in traditional PCR, there would be sequencing from both ends of the DNA at the same time and overlapping data would be produced.
5. **D**. See lecture slides on Maxam-Gilbert Sequencing.
6. **B**. See lecture slides on NGS. Note that to RNA sequencing first requires cDNA synthesis.
7. **C**. PacBio SMRT sequencing is more suitable for whole genome sequencing due to long reads and can simultaneously be used to identify epigenetic modifications throughout the genome, as seen in the table. Illumina sequencing is better for detecting mutations due to its cost efficiency for large amounts of samples in a population and high accuracy in its reads.
8. **D**. **c.78C>G** results in an amino acid change in a catalytic histidine residue in the active site, which may abolish the protein's enzymatic activity. **c.150ins** results in a frameshift mutation early in the sequence which will result in a nonfunctional protein and likely an early stop codon. **c.312A>C** is unlikely to affect the protein function much since it is a synonymous (not affecting amino acid sequence) mutation and does not affect the splice sites in the introns.

True-False Statements

1. **TTTT**. **A**: Illumina sequencing reconstructs genomes by sequencing short reads and aligning them bioinformatically, but repetitive sequences may be ambiguously aligned. **B**: True based on table. Higher raw error rates can be inferred due to the nature of sequencing longer reads. **C**: Bisulfite treatment converts unmethylated cytosines into uracil, allowing differentiation between methylated and unmethylated regions. **D**: True based on table.
2. **TFTF**. **A**: Conserved regions are likely functionally important while non-conserved regions are less significant. **B**: A glycine-to-alanine substitution is a conservative mutation since both are relatively small and hydrophobic and will not necessarily destabilise the structure. **C**: To amplify the entire exon for analysis, it should include sequences complementary to introns since purely exonic primers may exclude sequences adjacent to exons which are important for analysis. **D**: It may alter mRNA stability or affect splice sites.
3. **TTFT**. **A**: RFLP relies on variability in RE sites to generate different fragment patterns among individuals. **B**: Self-explanatory. **C**: Large-scale structural variations often do not produce interpretable fragment patterns. As such, RFLP is more suited for point mutation detection. **D**: See RFLP lecture in slides.
4. **TFTF**. **A**: Self-explanatory; see lecture for restriction mapping. **B**: Double digestion may provide more information, but this is not always the case; single digestion may be sufficient in some cases such as confirming the presence of a single restriction site. **C**: See restriction mapping lecture. **D**: When digesting with a single restriction enzyme, insertion of the insert in the wrong orientation can occur 50% of the time.
5. **FFTF**. **A**: Identical STR profiles indicate a match between the sample DNA and the target DNA; however, it does not prove guilt as other factors such as sample contamination could account for that. **B**: Monozygotic twins have the same STR profile as they have the exact same genome. **C**: Self-explanatory, as mutations may occur that interfere with STR analysis. **D**: The converse is true, since with less STR loci analysed there is a higher probability of a chance similarity which decreases with more STR loci analysed.
6. **FTFF**. **A**: DNA polymerase will not recognise the RNA template and qPCR cannot proceed. **B**: An amplification efficiency close to 100%, meaning that DNA amount doubles with each cycle, ensures that valid comparisons can be made between qPCR samples. **C**: Only relative changes can be estimated, not absolute changes in RNA levels. Absolute quantitation requires a standard curve with known concentrations. **D**: SYBR Green binds to double-stranded DNA.
   FTFF. See the lecture slides for explanations for the above.

IBO Questions

Please email us at contact@learntuitive.com specifying your request for the explanations for the IBO papers. Alternatively, you may request an answer on our Discord server (https://discord.gg/S3JNkt3wQ9).

1. **1. 920**µg/ml
   2. 2.17µl
   3. 9
   4. UV-B → G → B → D → UV protection
2. **TTTF**
3. **A. 1 day**
   B. 5000
   C. Primer 1b – **100bp**, Endonuclease cut site – **300bp**, Primer 2 – **1000bp**
   D. TFTT
4. **1. D**

Q.7.2	A	B	C	D	E	F	G
GG	x				x		
AG			x				x
AA		x		x		x	
	1	1	1	1	1	1	1

5. Refer to the table below

a	b	c	d	e	f
✓	✓	✓	✗	✓	✗

6. Refer to the table below

Lowest Tm	Medium Tm	Highest Tm
a	c	b

7. Refer to the tables below

	-35	-34	-33	-32	-31	-30	-29	
5’	T	A	T	A	A/T	A	A/T	3’

Sequence element				
Operator	Promoter	Origin of replication (ORI)	Telomere	Enhancer
×	✓	×	×	✓

8. Refer to the tables below

Q.6.1	**50**	2

	5’-3’	3’-5’	
Q.6.2	**X**		1

Q.6.3	**1.25**	3

Q.6.4	**L**	2

	A	B	C	D	
Q.6.5				**X**	2

9. FFTF

References

Multiple Choice Questions

1. Self-produced.
2. Self-produced.
3. Self-produced.
4. Self-produced.
5. Self-produced.
6. Self-produced.
7. Self-produced.
8. Self-produced.

True-False Statements

1. Self-produced.
2. Self-produced.
3. Self-produced.
4. Self-produced.
5. Self-produced.
6. Self-produced.
7. Self-produced.

IBO Questions

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1. IBO 2019 Theory 1 Question 9
2. IBO 2013 Theory 1 Question 10
3. IBO 2017 Theory 1 Page 17
4. IBO 2019 Theory 2 Question 7 Parts (1) and (2)
5. IBO 2012 Theory 1 Question 1
6. IBO 2012 Theory 1 Question 3
7. IBO 2012 Theory 2 Question 4
8. IBO 2019 Theory 1 Question 6
9. IBO 2020 Theory 1 Question 17