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|  | P:\Logos\BioDiscovery\BioDiscovery Logo Col Pos.jpg  P:\Logos\Perkins logos\Harry Perkins logos all formats\Logos jpeg\PI_L_COL.png**Validation Test: Techniques in Biotechnology** | | |
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**Name:**

**Part A: /6**

**Part B: /44**

**Total: /50**

### Time allowed for this paper

Working time for paper: 40 minutes

Structure of Paper

Part A: Practical component

Part B: Validation test including questions from excursion to the Harry Perkins Institute of Medical Research

### Material required/recommended for this paper

# To be provided by the supervisor

Question/answer booklet

# To be provided by the candidate

Standard items: Pens, pencils, eraser or correction fluid, ruler, highlighter

Special items: Scientific calculator

# *Important note to candidates*

No other items may be taken into the examination room. It is **your** responsibility to ensure that you do not have any unauthorized notes or other items of a non-personal nature in the examination room. If you have any unauthorized material with you, hand it to the supervisor **before** reading any further.

**PART B: (44 Marks)**

This section has nine (9) questions. Answer all questions. Write your answers in the spaces provided in this Question/Answer Booklet. Use a blue or black pen for this section.

Suggested working time: 40 minutes.

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**Question 1**

What is the function of each of the following in gel electrophoresis of DNA?

a) Agarose gel: (1 mark)

The agarose gel provides a matrix with pores to allow molecules to travel through and be sorted by size.

b) Electric current: (1 mark)

The electric current is the force that causes the negatively charged DNA molecules to more toward the positive pole.

c) "Wells" in the gel: (1 mark)

The wells are the "starting gates" for the DNA molecules to be loaded into before starting the "race".

**Question 2**

a) Toward which pole (positive or negative) does DNA migrate when electric current is run through the gel? (1 mark)

positive

b) Why do the DNA molecules move toward this pole? (1 mark)

The DNA molecues are negatively charged (opposite charges attract one another).

**Question 3**

What would happen to the DNA fragments if you forgot to turn the current off? (1 mark)

The DNA fragments would keep on running through the gel until they ran off the end.

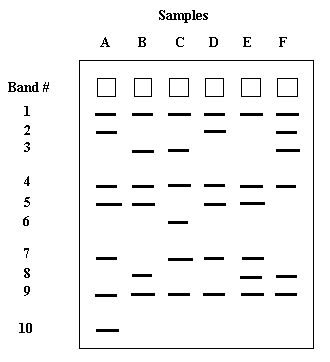
**Question 4**

Describe how different sized DNA fragments are separated by the gel matrix. (1 mark)

Longer DNA fragments take longer to work their way through the pores of the gel matrix, they don't travel as far through the gel as the shorter fragments in the same amount of time.

**Question 5**

Examine the diagram of an agarose gel below and answer the following questions.



a) What do the bands in the drawing of the agarose gel represent? (1 mark)

Many DNA fragments which are the same size

b) Which band(s) traveled slowest? (1 mark)

The bands nearest the wells (containing the longest DNA fragments) travelled the slowest. (Band #1)

c) Which band(s) travelled fastest? (1 mark)

The bands furthest from the wells (containing the shortest DNA fragments) travelled the fastest. Bands #10.

d) On the above drawing, label the positive and negative ends of the gel. (2 marks)

The negative pole is located closest to the wells. The positive pole is located further from the wells.

e) How many bands are shared in common by all of the individuals? (1 mark)

3 (Bands #1, #4 and #9)

f) Are there any bands which are unique to only one individual? If so, which one/s? (2 marks)

yes (1)

Bands #6 (sample C) and #10 (sample A). (1)

**Question 6**



Orangutans are an endangered species of ape that live in Southeast Asia. Their numbers have dwindled due to habitat loss and poaching, but there are currently efforts to save the orangutans.

DNA fingerprinting has been used by Dr. Benoit Goossens at Cardiff University to better understand mating and genetic variation in orangutans.

Use DNA fingerprinting (see below) to determine which male orangutan is the father of a baby orangutan. You will have DNA from the baby orangutan and several possible father orangutans. It is your job to determine which is the father .

In the BIO-RAD DNA Fingerprinting scenario which follows, each DNA sample stands for a different suspect, here (orangutan parentage scenario) each DNA sample stands for an individual orangutan. The picture below shows the results you would expect from the DNA Fingerprinting practical outlined here.

Mother baby Male 1 Male 2



1. Who is the father of the baby orangutan? ( 1mark)
2. (a) Male 1
3. Explain how you reached this conclusion (2 marks)

(b) Compare the bands of baby , mother and the two males. Male 1 had more bands that the baby had. ( note all had the top band so can ignore)

**Question 7**

**a)** Explain what is meant by DNA profiling/fingerprinting. (2 marks)

DNA profiling is the process whereby the unique pattern of each person’s DNA can be identified. (1) Profiling can be carried out using the process of electrophoresis.

It is different from DNA sequencing as this is the process of establishing the nucleotide (base) sequence of DNA for different species. (1)

b) List 2 fields that would use PCR in their work on a regular basis (other than medical). (2 marks)

(b) Crime investigators, food analysis.

**Question 8**

a) What is PCR? What role does electrophoresis play in this process? (3 marks)

PCR (Polymerase Chain Reaction) (1) is a technique that involves producing large quantities of DNA identical to a sample provided. (1) Electrophoresis is used to separate out the fragments of DNA that has been amplified for comparison with other of that species or closely related species (1)

b) What is a primer?

A short strand of DNA that is complementary to part of the target gene. They are essential for DNA polymerase to attach to DNA

c) What is Taq polymerase and what does it do? (2 marks)

It is an enzyme that adds nucleotides to the end of the primer and builds a complementary strand of DNA

d) List the three stages in PCR and describe what occurs at each stage. (6 marks)

1. denaturation: high temperature that causes double stranded DNA to denature (separate into single strands);

2. annealing: cooling that allows primers to anneal (bind) to DNA strands; and

3. elongation: increased temperature that allows Taq polymerase to add nucleotides, to build new DNA strands.

e) Why did you place a water sample in the thermocycler? (1 mark)

To act as a control

f) Name four of the reagents in the Master Mix (2 marks)

Buffer,

Mg Cl2

dNTP mix

BRAF forward primer

BRAF reverse primer

Taq polymerase

Water

*½ mark per correct answer*

g) What is a DNA ladder, and why is it used? (2 marks)

A sample containing DNA fragments of known size, enabling you to estimate the size of your DNA product

**Question 9**

a) What is BRAF and what does it do? (2 marks)

Gene found in normal cells that codes for a protein that is part of a cell signaling pathway in cell division

b) What is the significance of the mutated BRAF gene in melanoma? (2 marks)

The protein is continuously active and causes cells to continuously grow and divide.

Over 50% of patients with melanoma have the mutated BRAF gene

END OF TEST