**SOUTHERN RIVER COLLEGE**

**Human Biological Science**

**Unit 3 & 4**

**TASK 10**

**Harry Perkins Excursion Validation**

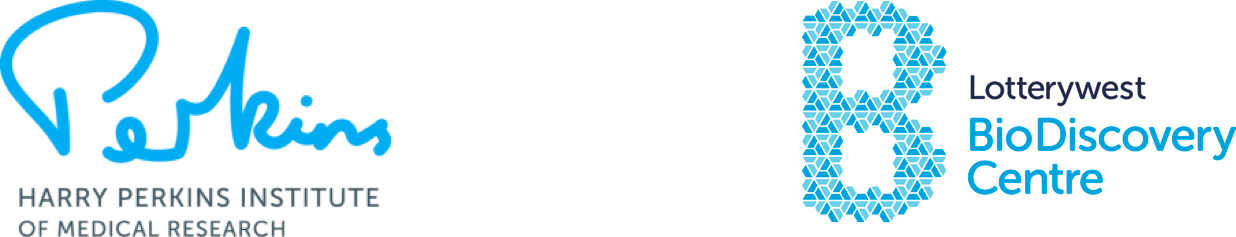
**Techniques in Biotechnology**

**MARKING KEY**

**Time: 60 mins**

**Weighting: 5%**

**Do not turn page until instructed to do so**



**(55 Marks)**

This section has ten (10) 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.

**Question 1**

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

a) Agarose gel: (2 marks)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Pores allow the molecules of DNA to travel through it | 1 |
| Sorts the pieces by size | 1 |

b) Electric current: (1 mark)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Causes the negatively charged DNA to move towards the positive pole  (Must have both) | 1 |

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Location where the DNA molecules are loaded into | 1 |

**Question 2**

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Positive | 1 |

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| The DNA is negatively charged  (opposite charges attract one another). | 1 |

**Question 3**

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| The DNA fragments would run off the end of the gel | 1 |

**Question 4**

Describe how different sized DNA fragments are separated by the gel matrix. (2 marks)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Longer DNA pieces take longer to get through the gel due to higher resistance | 1 |
| Shorter fragments travel faster due to less resistance  (must compare long to short) | 1 |

**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)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| DNA fragments which are (approximately) the same length of base pairs | 1 |

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Band number 1 | 1 |

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Band number 10 | 1 |

d) On the above drawing, label the positive and negative ends of the gel. (1 mark)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Negative pole closest to the wells and the positive pole further from the wells | 1 |

e) How many bands are shared in common by all of the individuals? List the common bands.

(2 marks)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| 3 bands | 1 |
| Bands 3, 5 and 7 | 1 |

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Yes | 1 |
| Band 2 (Sample F) and band 8 (sample B) | 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 .

Mother baby Male 1 Male 2

(a) Who is the father of the baby orangutan? (1 mark)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Male 1 | 1 |

(b) Explain how you reached this conclusion (2 marks)

Any 2 of the following:

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Identify which bands of the baby where from the mother | 1 |
| Remainder of baby’s bands were from male 1 | 1 |
| Compare the bands of the baby, mother and two males | 1 |

**Question 7**

**a)** Explain what is meant by DNA profiling/fingerprinting, and how it is different to DNA sequencing?

(2 marks)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| DNA profiling: process of using gel electrophoresis to identify the pattern of DNA | 1 |
| DNA sequencing: establishing the exact base (nucleotide) sequence of DNA for the sample | 1 |

Must have all.

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

Crime investigators/forensics, food analysis, anthropology (any 2 fields)

**Question 8**

a) What does PCR stand for, and what is its purpose? What role does electrophoresis play in this process? (3 marks)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Polymerase chain reaction | 1 |
| Produces large quantities/amplifies/copies DNA samples | 1 |
| Gel electrophoresis: separates the fragments out of DNA for comparison | 1 |

b) What is a primer? (3 marks)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Short strand of DNA complementary to the target DNA/gene | 1 |
| Identifies the start of the gene/DNA strand | 1 |
| Enables Taq/DNA polymerase to attached | 1 |

Must have all.

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Enzyme which adds nucleotides to the primer | 1 |
| and builds a complementary strand of DNA | 1 |

Must have all.

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| 1. Denaturing | 1 |
| 90-95 degrees causes the DNA strand to separate into single strands | 1 |
| 1. Annealing | 1 |
| 60 degrees allows primers to bind to the DNA strands | 1 |
| 1. Elongation/Extension | 1 |
| 72 degrees allows Taq polymerase to add nucleotides (builds new DNA strand) | 1 |

Must have all.

e) Why did you place a water sample in the thermocycler? (2 marks)

|  |  |
| --- | --- |
| **Description** | **Marks** |
| To add as a control | 1 |
| To check for cross contamination | 1 |

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Buffer,  Mg Cl2 (Cofactor)  dNTP mix  BRAF forward primer  BRAF reverse primer  Taq polymerase  Water | ½ per correct answer |

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| DNA fragments of a known size (in base pairs) | 1 |
| Enables you to estimate the size of DNA product | 1 |

**Question 9**

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Codes for a protein | 1 |
| essential in cell division (mitosis) | 1 |

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

|  |  |
| --- | --- |
| **Description** | **Marks** |
| Protein is continuously active | 1 |
| Causes cells to continuously grow and divide | 1 |

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

**Question 10**

Experiments are being conducted where mice are injected with altered genes. This could enable individuals with the mutated BRAF gene, to undergo gene therapy, replacing the faulty gene with a healthy one.

a) Identify the process used to create the altered gene which is being injected into the mice.

(1 mark)

|  |  |
| --- | --- |
| **Identify** | **Marks** |
| Recombinant DNA | 1 |

b) Name and describe the role of the two enzymes involved in the process you identified in part a). (6 marks)

|  |  |
| --- | --- |
| **Name + Describe** | **Marks** |
| **Restriction enzyme** | 1 |
| Isolates a gene (or section of DNA) at a recognition site | Any 2 |
| Plasmid/phage/bacteriophage/virus phage is cut with the SAME restriction enzyme  (must say one) |
| The cut produces sticky ends/staggered/jagged ends |
| **DNA ligase** | 1 |
| Joins the isolated gene/DNA segment and plasmid together | 1 |
| Joins at the sticky/staggered ends (of each) | 1 |

END OF TEST