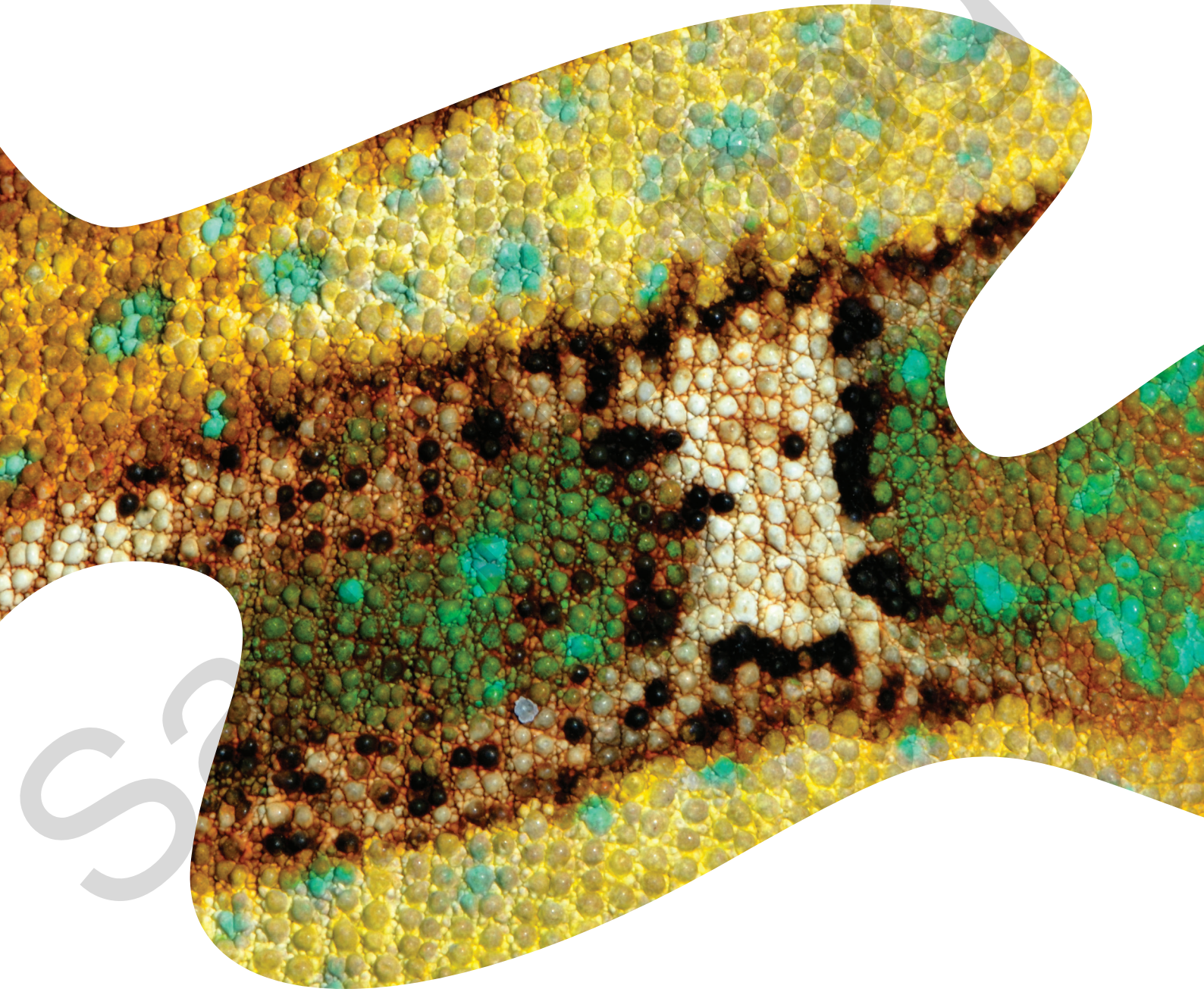
HEINEMANN

BIOLOGY 2

SKILLS AND ASSESSMENT



Yvonne Sanders

VCE UNITS 3 AND 4 • 2022-2026

Contents

Contents

 ISBN 978 0 6557 0026 5

AREA OF STUDY 2

How are species related over time?

AREA OF STUDY 3

How is scientific inquiry used to investigate cellular processes and/or biological change?

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How to use this book

The *Heinemann Biology 2 Skills and Assessment* book provides the opportunity to practise, apply and extend your learning through a range of supportive and challenging activities. These activities reinforce key concepts and skills and enable a flexible approach to learning. There are also regular opportunities for reflection and self-evaluation in the final worksheet in each area of study.

This resource has been written to the VCE Biology Study Design 2022–2026 and is divided into five areas of study—two in Unit 3 and three in Unit 4.

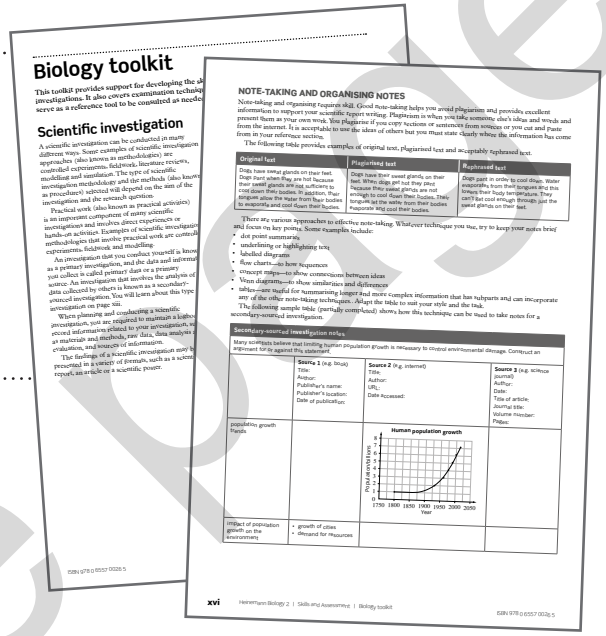
The first four areas of study consist of four main sections:

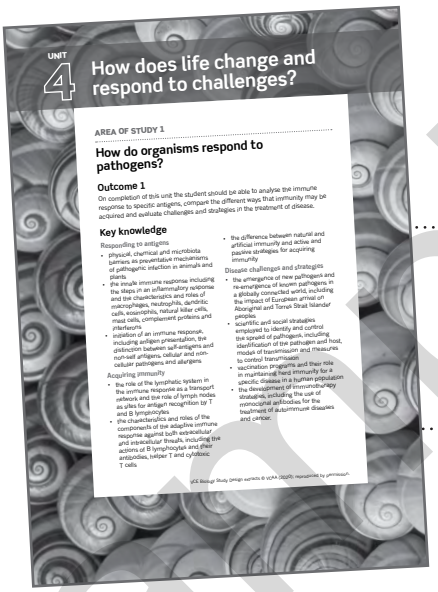
- key knowledge
- worksheets
- practical activities
- past VCE exam questions.

Area of Study 3 in Unit 4 supports development of the key science skills that you need to successfully design and conduct a scientific investigation.

BIOLOGY TOOLKIT

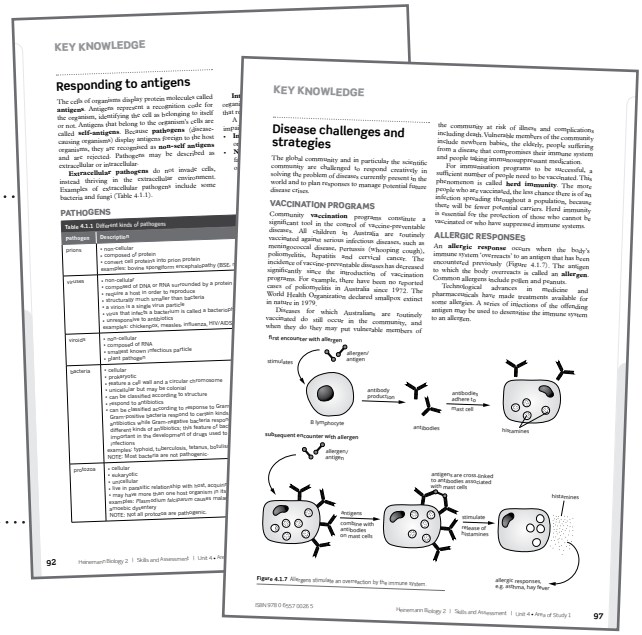
The Biology toolkit supports development of the skills and techniques required to undertake primary- and secondary-sourced investigations, and covers examination techniques and study skills. It also includes checklists, models, exemplars and scaffolded steps. The toolkit can serve as a reference tool to be consulted as needed.





UNIT AND AREA OF STUDY OPENER

Heinemann Biology 2 Skills and Assessment is structured to follow the study design units and areas of study. The area of study opening page lists the key knowledge for easy reference to the activities that follow.



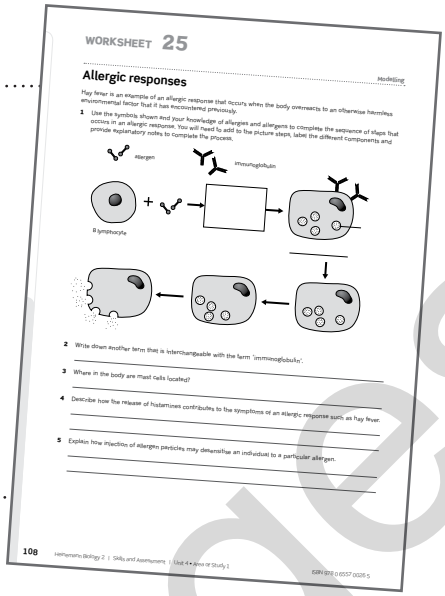
KEY KNOWLEDGE

Each area of study begins with a key knowledge section. This consists of a set of summary notes that cover the key knowledge for that area of study. Key terms are in bold and are included in the glossary of the student book. The section also serves as a ready reference for completing the worksheets and practical activities.

WORKSHEETS

The worksheets feature questions that allow you to practise and apply your knowledge and skills. Each area of study includes a 'Knowledge review' worksheet, to activate prior knowledge, and a 'Reflection' worksheet, which you can use for self-assessment. Other worksheets provide opportunities to revise, consolidate and further your understanding.

All worksheets function as formative assessment and are clearly aligned with the study design. A range of questions building from foundation to challenging is included in each worksheet.


WORKSHEET 25

Allergic responses

Modelling

Hay fever is an example of an allergic response that occurs when the body overreacts to an otherwise harmless environmental factor that it has encountered previously.

1 Use the symbols shown and your knowledge of allergies and allergens to complete the sequence of steps that occurs in an allergic response. You will need to add to the picture steps, label the different components and provide explanatory notes to complete the process.

allergen

immunoglobulin

B lymphocyte

2 Write down another term that is interchangeable with the term 'immunoglobulin'.

3 Where in the body are mast cells located?

4 Describe how the release of histamines contributes to the symptoms of an allergic response such as hay fever.

5 Explain how injection of allergen particles may desensitise an individual to a particular allergen.

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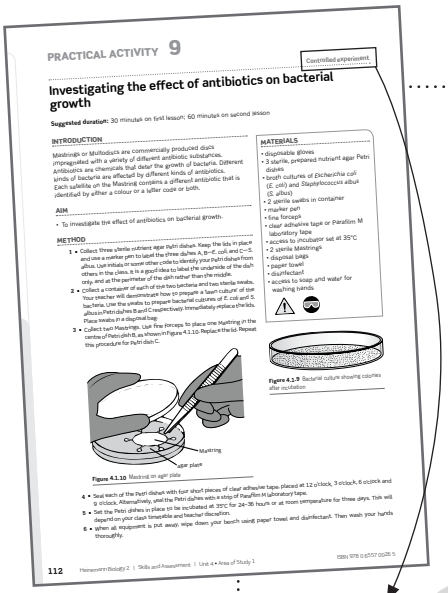
PRACTICAL ACTIVITY 9

Controlled experiment

Investigating the effect of antibiotics on bacterial growth

Suggested duration: 30 minutes on first lesson; 60 minutes on second lesson

INTRODUCTION

Mastrings® or Multidiscs are commercially produced discs impregnated with a variety of different antibiotic substances. Antibiotics are chemicals that delay the growth of bacteria. Different kinds of bacteria are affected by different kinds of antibiotics. Each safelilla on the Mastring contains a different antibiotic that is identified by either a colour or a letter code or both.

AIM

• To investigate the effect of antibiotics on bacterial growth.

METHOD

1 • Collect three sterile nutrient agar Petri dishes. Keep the lids in place and use a marker pen to label the three dishes A, B—E. coli and C—S. albus. Use initials or some other code to identify the Petri dishes from others in the class. It is a good idea to label the underside of the dish only, and at the perimeter of the dish rather than the middle.

2 • Collect a container of each of your two bacteria and two sterile swabs. Your teacher will demonstrate how to prepare a 'lawn' culture of one bacteria. Use the swabs to prepare bacterial cultures of E. coli and S. albus in Petri dishes B and C respectively. Immediately replace the lids. Place swabs in a disposal bag.

3 • Collect two Mastrings. Use fine forceps to place one Mastring in the centre of Petri dish B, as shown in Figure 4.1.10. Replace the lid. Repeat this procedure for Petri dish C.

MATERIALS

• disposable gloves
• 3 sterile, prepared nutrient agar Petri dishes
• broth cultures of Escherichia coli (E. coli) and Staphylococcus albus (S. albus)
• 2 sterile swabs in container
• marker pen
• fine forceps
• clear adhesive tape or Parafilm M laboratory tape
• access to incubator set at 35°C
• 2 sterile Mastrings
• disposal bags
• paper towel
• disinfectant
• access to soap and water for washing hands

Figure 4.1.9 Bacterial culture showing colonies after incubation

Mastring

agar plate

Figure 4.1.10 Mastring on agar plate

4 • Seal each of the Petri dishes with four short pieces of clear adhesive tape, placed at 12 o'clock, 3 o'clock, 6 o'clock and 9 o'clock. Alternatively, seal the Petri dishes with 4 strips of Parafilm M laboratory tape.

5 • Set the Petri dishes in place to be incubated at 35°C for 24–36 hours or at room temperature for three days. This will depend on your class timetable and teacher discretion.

6 • When all equipment is put away, wipe down your bench using paper towel and disinfectant. Then wash your hands thoroughly.

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Controlled experiment

PRACTICAL ACTIVITIES

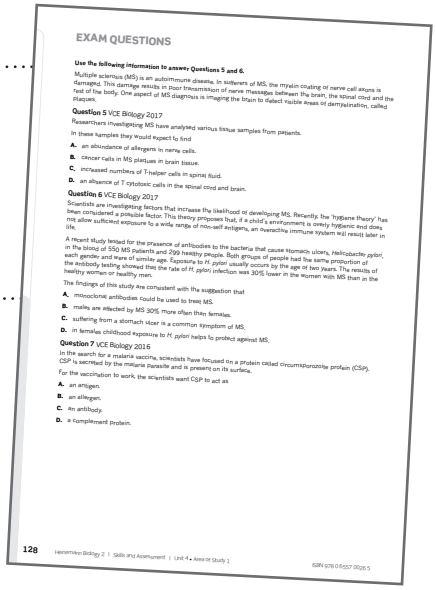
Practical activities offer you the chance to complete practical work related to the various themes covered in the study design. You have the opportunity to design and conduct scientific investigations, generate, evaluate and analyse data, appropriately record results and prepare evidence-based conclusions. Where relevant, you will also need to conduct risk assessments to identify any potential hazards.

Each practical activity includes a suggested duration. Together with the Area of Study 3 practical investigation, the practical activities meet the 30 hours of practical work mandated for Units 3 and 4 in the study design.

Each worksheet and practical activity is mapped to one or more of the scientific investigation methodologies outlined in the study design. Completing these activities gives you experience in applying the methodologies in a wide variety of contexts and prepares you for designing and conducting your own scientific investigation in Unit 4 Area of Study 3.

EXAM QUESTIONS

Each area of study finishes with a selection of past VCE Biology exam questions. This gives you the opportunity to draw together your knowledge and understanding, and to gain valuable experience applying this to actual exam questions.


EXAM QUESTIONS

Use the following information to answer Questions 5 and 6.

Multiple sclerosis (MS) is an autoimmune disease. In sufferers of MS, the myelin coating of nerve cell axons is damaged. This damage results in poor transmission of nerve messages between the brain, the rational cord and the rest of the body. One aspect of MS diagnosis is imaging the brain to detect visible areas of demyelination, called plaques.

Question 5 VCE Biology 2017

Researchers investigating MS have analysed various tissue samples from patients.

In these samples they would expect to find

A. an abundance of allergens in nerve cells.
B. cancer cells in MS plaques in brain tissue.
C. increased numbers of T-helper cells in spinal fluid.
D. an influence of T cytotoxic cells in the spinal cord and brain.

Question 6 VCE Biology 2017

Scientists are investigating factors that increase the likelihood of developing MS. Recently, the 'hygiene theory' has been considered a possible factor. This theory proposes that, if a child's environment is overly hygienic and does not allow sufficient exposure to a wide range of micro-self antigens, an overactive immune system will result later in life.

A recent study tested for the presence of antibodies to the bacteria that cause stomach ulcers, *Helicobacter pylori*, in the blood of 550 MS patients and 299 healthy people. Both groups of people had the same proportion of each gender and were of similar age. Exposure to *H. pylori* usually occurs by the age of two years. The results of the antibody testing showed that the rate of *H. pylori* infection was 30% lower in the women with MS than in the healthy women or healthy men.

The findings of this study are consistent with the suggestion that

A. monoclonal antibodies could be used to treat MS.
B. males are affected by MS 30% more often than females.
C. suffering from a stomach ulcer is a common symptom of MS.
D. in females childhood exposure to *H. pylori* helps to protect against MS.

Question 7 VCE Biology 2016

In the search for a malaria vaccine, scientists have focused on a protein called circumsporozoite protein (CSP). CSP is secreted by the malaria parasite and is present on its surface.

For the vaccination to work, the scientists want CSP to act as

A. an antigen.
B. an allergen.
C. an antibody.
D. a complement protein.

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TEACHER SUPPORT

Comprehensive answers and fully worked solutions for all worksheets, practical activities and exam questions are provided via the *Heinemann Biology 2* <TBC>. In-depth support for Unit 4 Area of Study 3 in the form of samples, templates and teacher notes is also included, along with an interactive SPARKlab for every practical activity.

Biology toolkit

This toolkit provides support for developing the skills required to undertake scientific investigations. It also covers study skills and examination preparation. The toolkit can serve as a reference tool to be consulted as needed.

Scientific investigation

A scientific investigation can be conducted in many different ways. Some examples of scientific investigation approaches (also known as methodologies) are controlled experiments, fieldwork, literature reviews, modelling and simulation. The type of scientific investigation methodology and the methods (also known as procedures) selected will depend on the aim of the investigation and the research question.

Practical work (also known as practical activities) is an important component of many scientific investigations and involves direct experiences or hands-on activities. Examples of scientific investigation methodologies that involve practical work are controlled experiments, fieldwork and modelling.

An investigation that you conduct yourself is known as a primary investigation, and the data and information you collect is called primary data or a primary source. An investigation that involves the analysis of data collected by others is known as a secondary-sourced investigation. You will learn more about this type of investigation on page xv.

When planning and conducting a scientific investigation, you are required to maintain a logbook to record information related to your investigation, such as materials and methods, raw data, data analysis and evaluation, and sources of information.

The findings of a scientific investigation may be presented in a variety of formats, such as a scientific report, an article or a scientific poster.

CONDUCTING A SCIENTIFIC INVESTIGATION

Scientific investigations follow a precise scientific method. The checklist on the following page provides a summary of the elements that are common to many scientific investigation methodologies and scientific reports. Refer to the checklist and record important information as you conduct your scientific investigation.

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PRESENTING A REPORT ON A SCIENTIFIC INVESTIGATION

Scientific findings may be presented in a variety of ways. A common presentation format at science conferences is a poster. Posters can get ideas across to a large audience in an organised, concise and creative way. Other common presentation formats are essays, reports, oral presentations and articles. Each presentation format has its own conventions. The following table summarises the characteristics of a number of presentation formats.

Presentation format	Characteristics/inclusions	
poster	• balance of text and visuals • title, subheadings • balanced layout • captions for figures and tables	• references • hierarchy of font size according to subheading level • consistent font style—no more than three fonts
report/article	• structured with an introduction, paragraphs and conclusion • includes subheadings	• mainly text • can include diagrams, graphs and tables
essay	• structured with an introduction, paragraphs and conclusion • introduction states focus of essay • each paragraph makes a new point supported by evidence	• each paragraph links back to last paragraph • a text-style presentation format—visuals at end in appendix • conclusion draws all ideas together but does not include any new information
oral presentation	• needs to be engaging • refer to cue cards but do not read from them • watch audience as you speak	• stand still and avoid fidgeting • look at audience and appear confident

PROOFREADING

After you have completed the investigation and prepared your presentation, it is important to think about and check what you have done.

Proofread your work to minimise errors and maximise effective communication of the ideas from your investigation. Use the following questions as a proofreading checklist.

Proofreading checklist	Tick ✔
Have I:	
• investigated the question fully?	
• expressed myself clearly to communicate my ideas well?	
• used the scientific writing style?	
• included data analysis?	
• checked spelling, punctuation and grammar?	
• included references?	
• met the requirements of the presentation format?	

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Study skills

There are a variety of techniques and strategies you can use to help you study. You may find that you use different strategies in different situations. For example, you may prefer to highlight key phrases in your notebook throughout the year but make summaries of topics before an examination. The strategies you choose will depend on personal preference and may not be the same as those used by your classmates.

Effective study skills involve more than the learning strategies you use. Equally important is when you use those skills. It is more effective to apply study skills throughout the year, revising and consolidating your knowledge as you progress through the course, rather than doing a rushed cram just before the examination. Revise your work regularly. Being organised and setting up a study plan is key to reducing your stress.

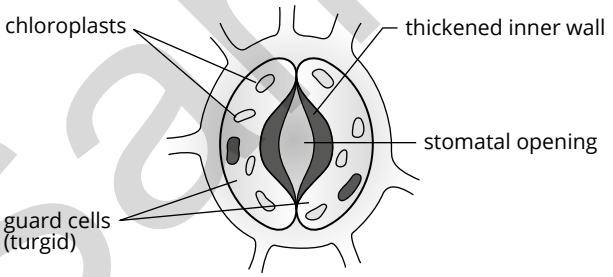
GETTING ORGANISED

To get yourself organised, try the following steps.

- Use a diary to write down all homework and assessment tasks as soon as you get them. Note due dates and what is required.
- Be specific about the tasks you need to do. Rather than writing 'do biology', it is more effective to note things such as which questions to answer and which page to look at in your student book.
- Write a list of everything you need to do each day. Tick off or cross out items as you complete them.
- Break down larger tasks into smaller separate parts that are manageable.
- Make sure your lists and planners are realistic. Do not set yourself more than you can actually do.

STUDY TECHNIQUES

Studying requires concentration. Remove any distractions and factor in some breaks. Allow a 10-minute break every hour. Vary your study technique depending on the content to be learned and your personal preference. Although you may have already found a study technique that works for you, also consider the following options.

Study technique	Tips
Highlighting FUNGI Fungi often look like plants but do not use photosynthesis. Instead they feed on dead and decaying material, breaking it down further and helping chemical elements to return to the natural environment. Mushrooms, toadstools, yeasts and moulds are different types of fungi.	• Highlight or underline key points as you read your notes or text.
Summary notes *ENZYMES* *Enzymes are:* • *composed of protein* • *substrate-specific* • *denatured by exposure to excessive heat* • *denatured by exposure to extremes of pH.*	• Create a list of key headings and add some dot points about each heading. • Write your own summary of the key ideas in each chapter. • Use headings and subheadings. • Underline key words and key phrases. • Use simple diagrams. • Remember, the most effective chapter summaries are clear, concise and uncluttered.
Diagrams  	• Diagrams can be used as a summary of key concepts. • Diagrams are useful memory triggers. • Diagrams cover a lot of information in a visual way, with minimal text.

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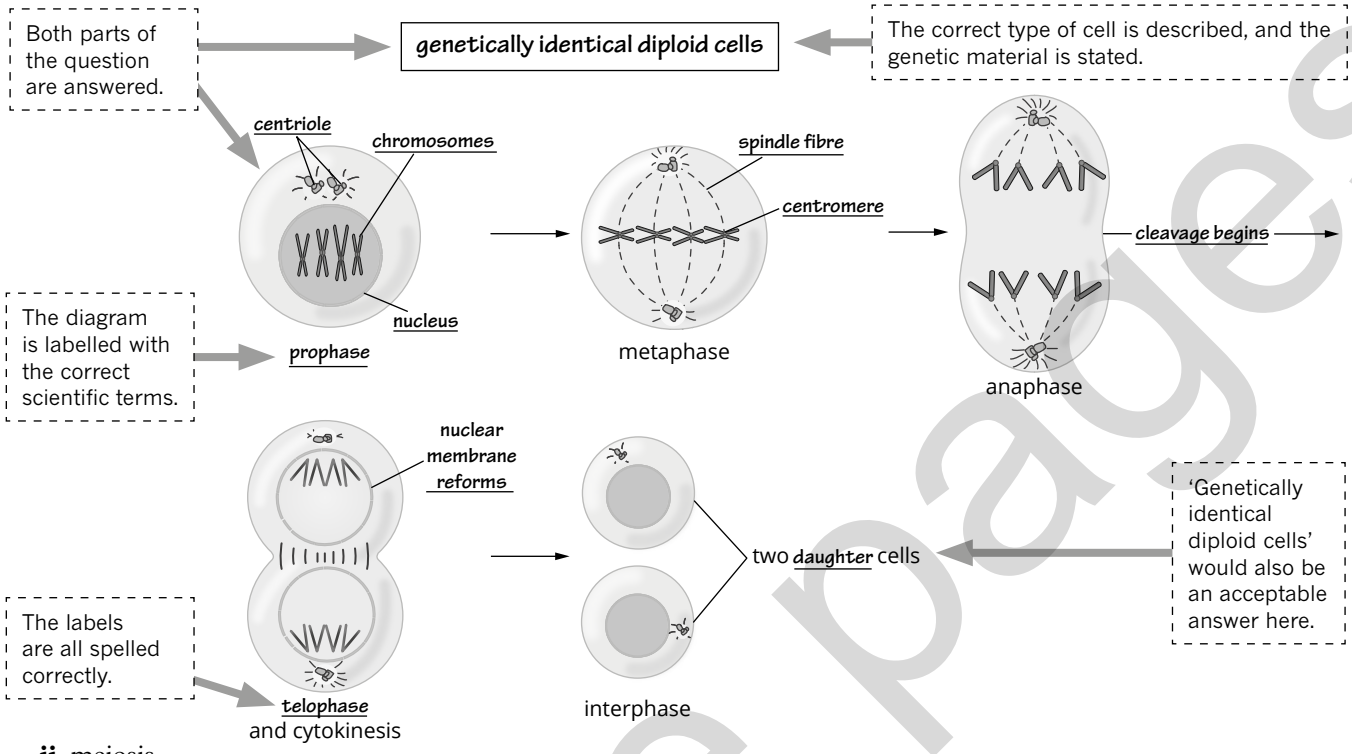
High-level response

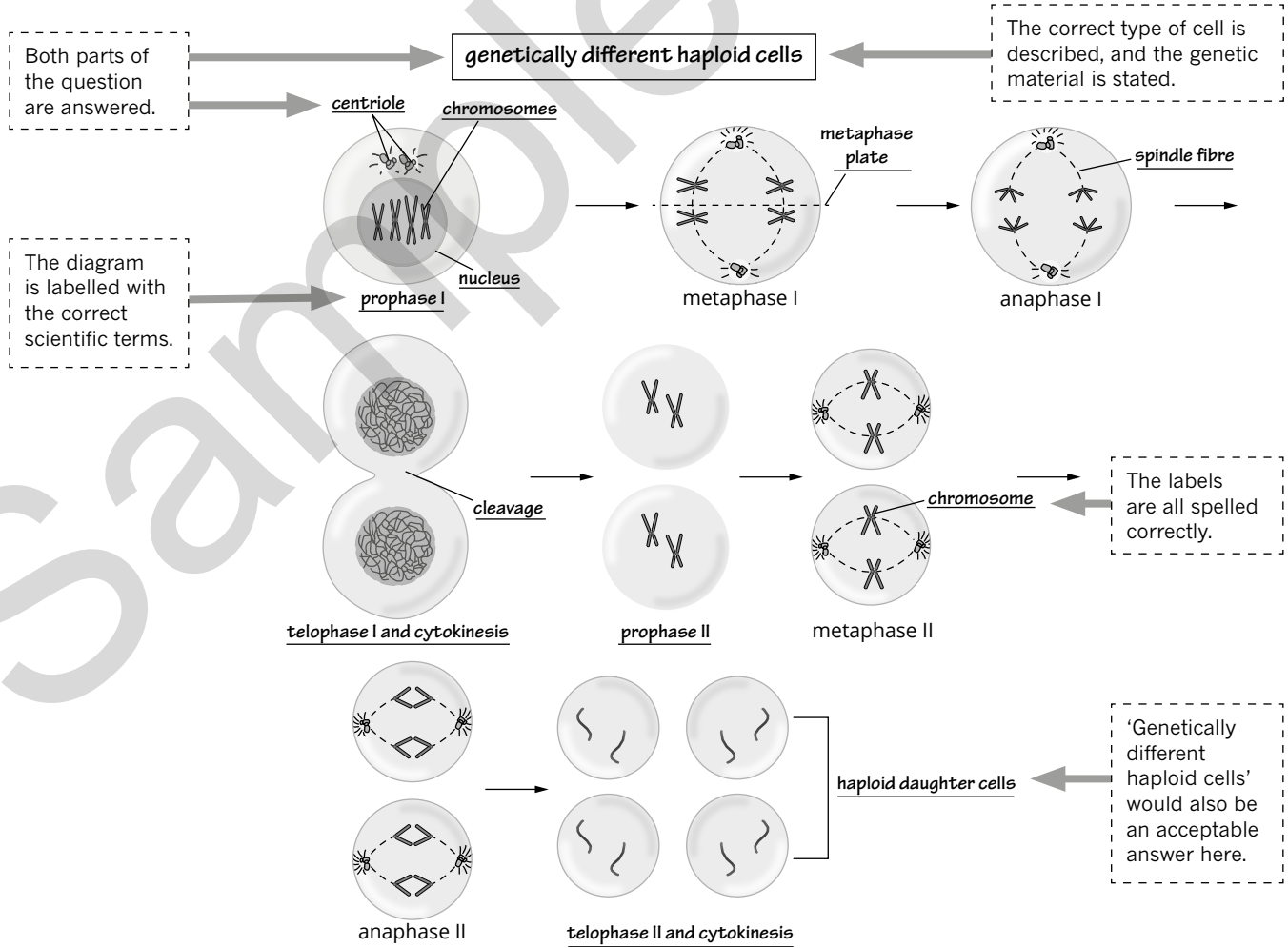
Question 1 (12 marks)

a DNA replication occurs during the synthesis phase of the cell cycle. This is followed by nuclear and cell division. Describe the type of cells that result from mitosis and meiosis, shown below, and complete the labels in the diagrams provided.

i mitosis



ii meiosis



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b Consider the image of two homologous chromosomes shown to the right.

Name the point at which these chromosomes cross over, and identify the stage named in part **a** in which this event occurs. Explain the significance of this event for the continuity of species.

- The point of crossing over is correctly named.
- The correct stage in which crossing over occurs is identified.
- Both parts of the question are answered.

Chiasma is the point at which homologous chromosomes cross over during prophase I in meiosis. Crossing over allows homologous chromosomes to exchange genetic material, producing new combinations of genetic information in the daughter cells. This process is called recombination. Crossing over is significant for the continuity of species because it produces genetic diversity, which allows populations and species to adapt to changing environmental conditions, improving their chance of survival and reproduction.

- The reasoning behind the answer is presented, demonstrating understanding of the content.
- Correct scientific terminology is used.
- The explanation provides clear information about the process and its significance for the continuity of species.

c Identify and explain:

i one disadvantage of asexual reproduction

- The disadvantage is stated and an explanation of why it is a disadvantage is given.
- The implications of the disadvantage are proposed.
- The limiting conditions are stated.

In a changing environment, for example where a disease is introduced, all individuals in an asexually reproducing population are vulnerable, meaning the population and the species could become extinct. Asexual reproduction is usually a disadvantage in the long term.

- An example is used to illustrate the explanation.
- The disadvantage is explained in terms of the individual, the population and the species.
- Relevant scientific terminology is used.

ii one advantage of sexual reproduction.

- The advantage is explained in terms of the individual, the population and the species.
- The limiting conditions are stated.
- Relevant scientific terminology is used.

Sexual reproduction generates genetically varied offspring, increasing the chance of adaptation and survival of the population and the species in changing environmental conditions. For example, genetic diversity in a population may mean that some individuals in that population are resistant to a new disease. Increased genetic diversity improves the evolutionary potential of a species.

- The advantage is stated and an explanation of why it is an advantage is given.
- The implications of the advantage are proposed.

UNIT 3

How do cells maintain life?

AREA OF STUDY 1

What is the role of nucleic acids and proteins in maintaining life?

Outcome 1

On completion of this unit the student should be able to analyse the relationship between nucleic acids and proteins, and evaluate how tools and techniques can be used and applied in the manipulation of DNA.

Key knowledge

The relationship between nucleic acids and proteins

- nucleic acids as information molecules that encode instructions for the synthesis of proteins: the structure of DNA, the three main forms of RNA (mRNA, rRNA and tRNA) and a comparison of their respective nucleotides
- the genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes
- the structure of genes: exons, introns and promoter and operator regions
- the basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of a regulatory process
- amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein
- proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways
- the role of rough endoplasmic reticulum, Golgi apparatus and associated vesicles in the export of proteins from a cell via the protein secretory pathway

DNA manipulation techniques and applications

- the use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA and endonucleases to cut DNA
- the function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome
- amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling
- the use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin
- the use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease.

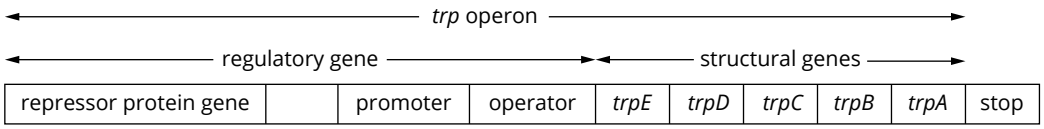

Figure 3.1.11 *Trp* operon

The ***trp* operon** (tryptophan operon) model in bacteria serves as a classic example of our understanding of gene function. An **operon** is a group of genes with a regulatory role in protein production. The *trp* operon refers to a bacterial gene responsible for the production of the amino acid tryptophan. When tryptophan levels are high, a repressor protein binds to the operator site of the regulatory gene, switching the *trp* operon off, and resulting in no further tryptophan production. When tryptophan levels are low, the repressor protein is released and the *trp* operon is switched on. Transcription proceeds, resulting in the production of an enzyme that produces tryptophan (Figure 3.1.11).

The expression of genes can be influenced by various environmental factors, such as temperature, light and pH.

Because all cells need to engage in life-sustaining functions such as cellular respiration, the genes that control these processes are switched on in all cells. Such genes are referred to as 'housekeeping' genes.

PROTEIN STRUCTURE

Once polypeptide production is complete, the final formation of the protein can occur (Table 3.1.6).

Table 3.1.6 Levels of protein structure

1	polypeptide formation	primary protein structure
2	polypeptide becomes coiled or pleated	secondary structure
3	coiled polypeptide folds into three-dimensional form	tertiary structure
4	two or more three-dimensional polypeptide molecules bonded together	quaternary structure

Proteins are large biomolecules that can contain thousands of amino acids and may be synthesised as one or several polypeptide chains. These polypeptide chains are folded and organised into specific shapes that are vital to the correct functioning of the protein (Figure 3.1.12).

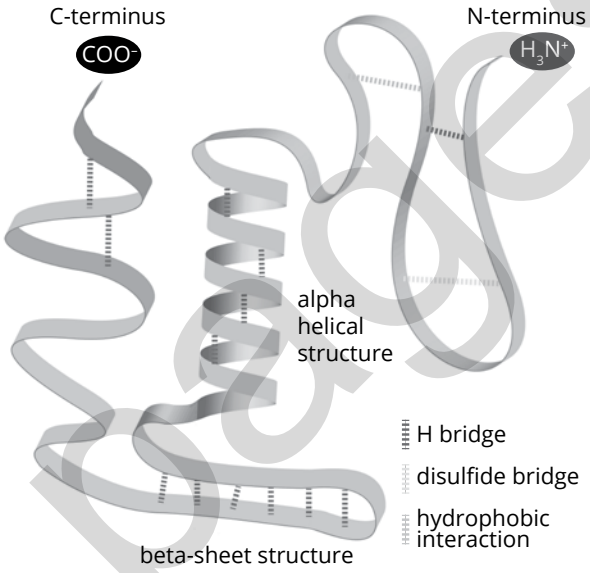

Figure 3.1.12 A simplified ribbon diagram showing segments of the primary or linear structure of a protein (polypeptide) folded into a beta sheet and alpha coil (secondary structures). The three-dimensional folding of the molecule represents the tertiary structure of the protein.

Proteins are key components of cells. There are many different kinds of proteins, each with a different function. For example, proteins in collagen have a structural role, while those embedded as plasma membrane channels have a transport role. Enzymes are of particular significance as they are involved in catalysing biochemical pathways that keep cells, and hence the entire organism, functioning. A multitude of enzymes are involved in the processes of cellular respiration and photosynthesis, as well as many other processes. Enzymes as catalysts are more fully addressed in Unit 3 Area of Study 2. Proteins in all their varied forms are vital to the normal functioning of organisms. Figure 3.1.13 illustrates some of examples of the diverse kinds of proteins in living things and their roles.

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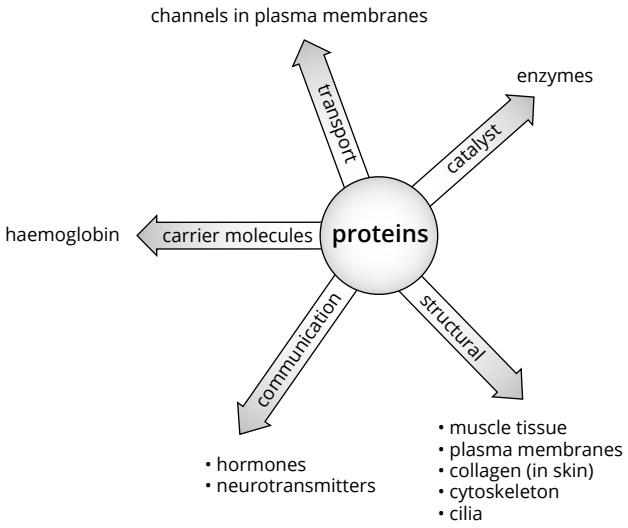

Figure 3.1.13 Proteins have many roles in living organisms.

PROTEIN SECRETORY PATHWAY

Secretory proteins are proteins that are produced to be exported from a cell. The movement of secretory proteins occurs by **exocytosis**, also known as the **protein secretory pathway**. Before reaching the plasma membrane for exocytosis, secretory proteins must first be synthesised and modified.

Proteins destined for use within the cell are synthesised by free ribosomes that are found in the cytosol. Proteins that are to be secreted are synthesised by ribosomes that stud the outer surface of the **rough endoplasmic reticulum**.

When a section of the plasma membrane wraps around a substance for import into the cell, pinching off to form a vesicle inside the cytoplasm, the process is called **endocytosis**. Pinocytosis refers to a similar process related to the import of liquid droplets. Exocytosis is the opposite of endocytosis and involves **vesicles**, such as those at associated with the **Golgi apparatus**, merging with the cell's plasma membrane to facilitate the export of substances such as proteins, for example, hormones.

THE PROTEOME

The proteome is the total complement of all of the proteins in an individual organism. An organism's proteome is determined by the DNA sequence of its genome. **Proteomics** (the study of proteins, including their structure and function) is an expanding field of biology that has enormous potential for increasing our understanding of how organisms function, of diseases and their treatment and management, for the development of pharmaceuticals, and for shedding light on evolutionary relationships.

Bioinformatics (the use of computers and databases to manage biological information) is a vital tool in collecting and analysing biological information, as well as making data accessible and manageable. For example, generating the DNA sequence of the human genome and making the data accessible are results of this technology.

DNA manipulation techniques and applications

Humans impact biological processes, including evolutionary processes, in all sorts of ways, from selectively breeding plants and animals to serve our needs, to applying cutting edge technological inventions from genetic screening to genetic modification of organisms. Such interventions make humans unique in their ability to alter the course of evolutionary change in their own and other species.

GENETIC TOOLS AND TECHNOLOGIES

Advances in genetic research have made a range of tools and techniques available for DNA manipulation. Such advances have important applications in:

- medicine—diagnosis of disease and development of pharmaceuticals
- forensics—drawing conclusions related to crime investigations from DNA analysis
- evolutionary biology—DNA analysis provides information about relationships between different kinds of organisms
- bioinformatics—gathering and manipulating biological data.

Genetic tools

- Gene probe: single-stranded DNA (or RNA) sequence that is complementary to a part of the target DNA sequence used to identify the location of a particular gene or DNA fragment. Gene probes are tagged (either radioactively or fluorescently) to make them easily identifiable after binding to a target DNA sequence.
- **Primer**: short, single-stranded sequence of DNA (or RNA) that is complementary to part of the target DNA. It binds to a section of DNA that has been targeted for amplification in polymerase chain reaction (PCR), thereby identifying target DNA.
- **DNA ligase**: enzyme that joins 'sticky ends' of cut DNA strands according to complementary base-pairing rules.
- **Reverse transcriptase**: enzyme used to build 'copy DNA' (cDNA) from mRNA template, that is, transcription occurs in reverse.

- **Restriction enzyme**: enzyme specialised for 'cutting' a DNA strand. Restriction enzymes feature a specific nucleotide sequence that is used to identify and isolate a target piece of DNA. This recognition sequence allows the restriction enzyme to bind to a 'recognition site' and severs ('cuts') the bond at a particular site in the DNA sequence (sometimes called 'molecular scissors').
  - If the restriction enzyme 'cuts' the two strands in the DNA molecule in precisely the same position, the ends are called 'blunt' (Figure 3.1.14). When the restriction enzyme cuts the two strands in different positions, bases on both strands become exposed and can bind to available complementary bases. These are called 'sticky ends' because they can rejoin (Figure 3.1.14).
  - Restriction enzymes are isolated from bacteria.
  - Restriction enzymes are also called **endonucleases**.

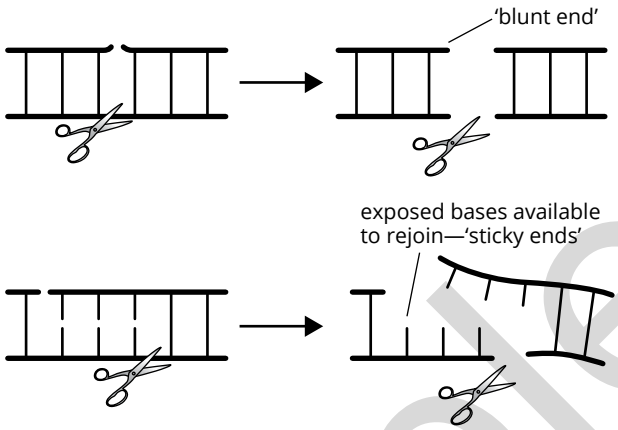

Figure 3.1.14 Restriction enzyme cuts DNA

Genetic technologies

Genetic modification describes the transfer of genes from one organism into the genome of another organism, usually of a different species. This means the organism has been genetically transformed, hence the term **genetic transformation**. Organisms that have their genetic material modified are called **genetically modified organisms (GMOs)**. Organisms that have been genetically modified so that a gene or genes from another species have been inserted into their DNA are called **transgenic organisms**. Bacterial **plasmids** are increasingly used as vectors to confer desirable characteristics into crop plants, for example, to increase crop yield and/or confer resistance to pests and disease. Canola, cotton and safflower represent crops subjected to these technologies in Australia.

CRISPR-Cas9 is a bacterial enzyme complex that allows for genome editing in living cells. CRISPR is an abbreviation for 'clustered regularly interspaced short palindromic repeats'. Essentially, short repetitive DNA sequences that form palindromes (read the same both forwards and backwards) occur in clusters with regular spacing between them. The spacers contain unique DNA segments (that is, not repeats). Scientists believe the unique DNA sequences are copies of viral DNA retained by the bacteria after infection and represent a kind of memory, allowing the bacteria to quickly produce RNA that is complementary to the CRISPR arrays (known as CRISPR RNA or crRNA). The bacteria use the crRNA to target the virus' DNA and cut it with endonuclease enzymes, disabling the virus. The enzyme that cuts the viral DNA is a CRISPR associated protein called Cas9. The CRISPR-Cas9 system allows the bacteria to combat subsequent exposures to the same virus more effectively. Scientists have developed a CRISPR-Cas9 system that works in a similar way in laboratory conditions, enabling them to edit DNA sequences in living cells. This genetic tool has far-reaching potential for altering the genomes of organisms to confer the most desirable characteristics, including crops and livestock as well as correcting faulty genes that are the cause of genetic disorders.

Gel electrophoresis compares the distance that specific DNA fragments travel along a gel preparation that is subject to an electric current (to drive the DNA through the gel) (Figure 3.1.15). Smaller DNA fragments move further through the gel compared to larger fragments. The DNA samples being analysed are compared to standards (DNA fragments of known length) to determine the length of the sample DNA. Length of DNA fragments are measured in 'base pairs' (bp).

DNA profiling, also called DNA fingerprinting, uses a pattern of repeated DNA sequences (called 'short tandem repeats'—STRs) that are unique to an individual to identify a particular person's DNA (Figure 3.1.16). The DNA profile is observed using gel electrophoresis technology. DNA profiling is used to establish relationships between individuals, for example, to determine paternity or to confirm or exonerate crime suspects.

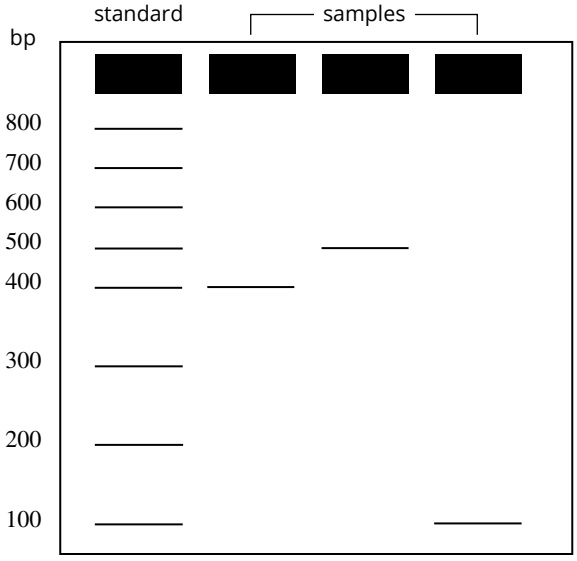

Figure 3.1.15 Gel electrophoresis

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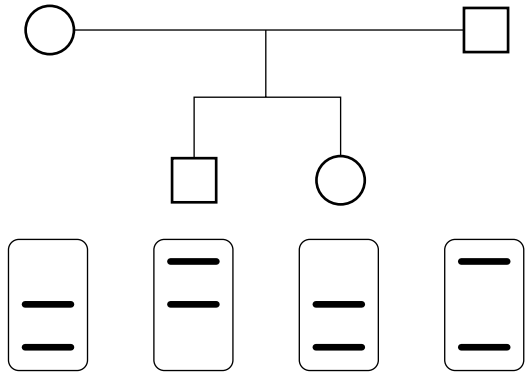

Figure 3.1.16 Family relationships evident using DNA profiling

Polymerase chain reaction (PCR) is a technique that amplifies or copies a target fragment of DNA. PCR makes large volumes of target DNA available for other applications, for example, gel electrophoresis and DNA profiling (Figure 3.1.17).

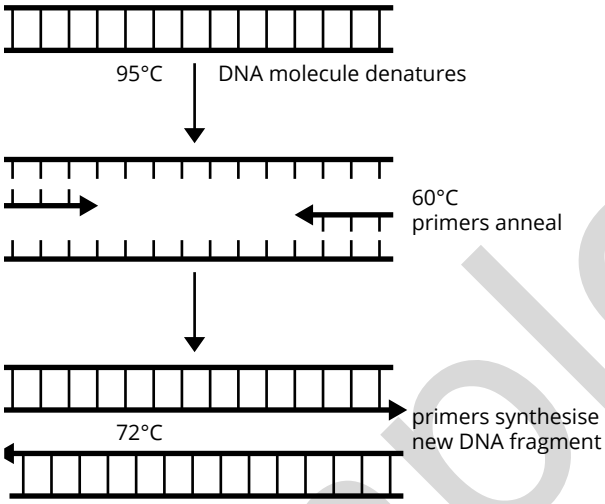

Figure 3.1.17 PCR amplifies a DNA fragment.

DNA sequencing is a process that is used to determine the order of nucleotide bases along a segment of DNA. Bases are 'tagged' so that each appears a different colour when viewed under fluorescent light. Chromatography is used to observe the tagged bases in a series of coloured peaks. The order of the coloured peaks reflects the order of the bases in the DNA strand (Figure 3.1.18). Identifying the base sequence of the human genome in the Human Genome Project is a classic example of the use of this technology. This technology is also used to compare DNA sequences from different species, establishing how closely related they are.

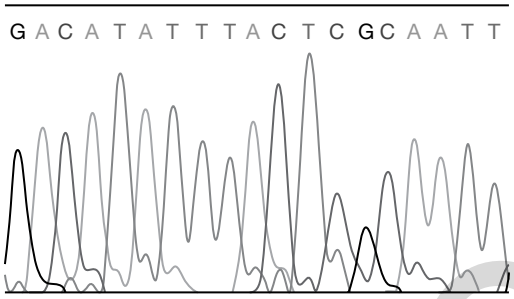

Figure 3.1.18 DNA sequencing. Each peak reflects a particular

Gene cloning involves making copies of a selected gene. This technology is applied in the genetic modification of bacterial DNA to construct **recombinant** plasmids. Such transgenic bacteria are important in the genetic modification of crop plants such as Bt cotton. Gene cloning also has medical applications, for example, in the production of human insulin for the treatment of diabetes.

Gene therapy is a process that imports a healthy gene into the DNA of a vector, such as a disabled virus, to treat diseases caused by the inheritance of defective genes. Cystic fibrosis is an example of such a genetic condition.

Cloning technology is also applied to create genetically identical individuals. Cloning of plants is easy to do from cuttings and has been a long-standing practice in agriculture. Animal cloning involves the removal of a diploid nucleus from a somatic cell and insertion into an emptied ovum that is ready for fertilisation. After a period of laboratory incubation, the developing embryo is implanted into the uterus of an adult female. A normal pregnancy ensues and the resulting offspring is a genetic clone of the donor of the somatic cell nucleus. Cloning is used in the production of crops and livestock with desirable characteristics, for example, pest resistant crops or increased crop yield. Therapeutic cloning is used to produce tissue that is suitable for transplant in humans, for example, to produce new skin for burns victims.

Genetic screening is a technique used to identify the presence or absence of genetic disorders, which may be caused by defective genes or abnormal chromosome numbers. This procedure is an important tool in assessing both the chance of developing the genetic disease in question or passing it on to potential offspring. As such, it provides information to assist decision-making.

Knowledge review—scientific method and genetic technologies

Scientific method

The scientific method is a vital tool that ensures a sound approach to investigations that yield reliable data and logical conclusions. By following the scientific method, researchers can contribute to the development of rigorous biological principles.

1 A student decided to test the idea that potatoes left in a dark cupboard can sprout stalks by vegetative reproduction. To test this idea the student placed 10 similar sized potatoes in a dark cupboard and another 10 potatoes on the kitchen bench for four weeks.

a Complete the table by entering the definition for each term and the example of each in this experiment.

Element of experiment	Definition	In this experiment
hypothesis		
independent variable		
dependent variable		

At the end of the test period the student observed that all of the potatoes in the dark cupboard had grown stalks, some short and some long, ranging from 2 to 6 cm in length. The potatoes placed on the kitchen bench also showed some growth, but the stalks were much shorter and there were fewer of them.

b Clarify which of the student's observations represent qualitative data and which represent quantitative data. Explain the difference.

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Genetic technologies

This part of the activity aims to remind you of some genetic technologies you have heard about before and to consider your understanding and perspective about them.

2 **a** Consider each of the technologies listed in the table below. Prepare a description outlining your understanding of the technology and write one or two questions the technology raises.

Genetic technology	Description	Questions
GM foods		
DNA profiling		
cloning		

b Discuss your responses with other members of the class. Add to your own list. Use online resources to refine your understanding in each case.

3 Scientific advances in genetic research have made technologies available that are important in medicine, forensics and evolutionary biology. Such technological advancements deliver advantageous outcomes for many, but also raise issues that are often the subject of debate in the community.

Select one genetic technology you have heard about and outline two benefits and two concerns.

Genetic technology: _____

a benefits

_____

_____

b concerns

_____

_____

Case study

Gel electrophoresis in species conservation

Figure 3.1.20 The Amur leopard, *Panthera pardus orientalis*

Gel electrophoresis has proved to be a critical tool in analysing genetic relationships in a range of applications, including familial relationships, forensic investigation and evolutionary relationships. It also has an important role in the conservation of species, particularly critically endangered species such as the Amur leopard, *Panthera pardus orientalis* (Figure 3.1.20). Dwindling wild populations continue to be threatened by habitat loss to make way for human population growth and the poaching of animals for their coats (Figure 3.1.21). With as few as 70 animals estimated to be living in the wild, breeding programs between zoos are vital in keeping them from extinction. Gel electrophoresis allows zoos to identify the closeness of the relationship between a potential breeding pair.

The gel electrophoresis shown in Figure 3.1.22 considers several DNA markers for a female Amur leopard and three potential mates from other zoos. Examine the completed gel run. Use the information to answer the questions.

Figure 3.1.21 Black indicates the only remaining wild populations of the Amur leopard.

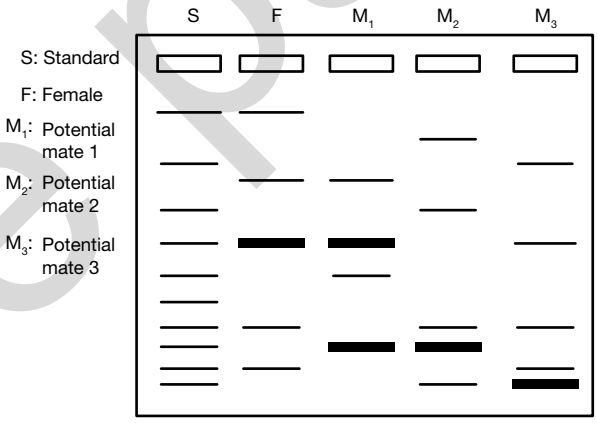

Figure 3.1.22 Gel electrophoresis

1 Identify the pair of Amur leopards you would recommend for breeding. Explain your choice.

2 One characteristic of many endangered species is small population size. What impact might this have on the gene pool of a species?

3 To what extent is a technique such as gel electrophoresis useful in captive breeding programs?

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Applications of genetic transformation

The year 2003 saw a novel direction in human intervention in the natural world. A new breed of designer pet appeared for sale in some countries—a glow-in-the-dark, genetically modified zebrafish (Figure 3.1.25).

The curious ornamental fish has been the subject of gene technologies that allow the gene in question to be isolated from jellyfish and then inserted into fish embryos at a very early stage of development—the one or two cell stage.

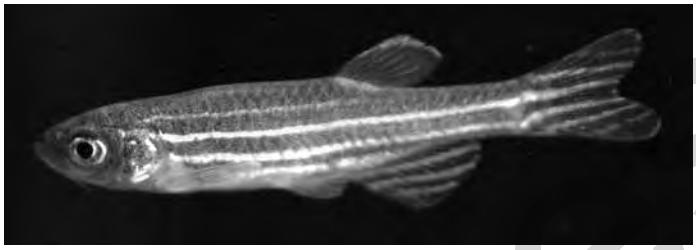

Figure 3.1.25 Transgenic fluorescent zebrafish (*Danio rerio*)

1 Use your knowledge of gene technologies to draw a flow chart of steps that occur in genetic transformations of this kind. Include genetic tools used in the technology. Diagrams might also be helpful.

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2 Suggest the likely fate of a transgenic fluorescent zebrafish if it were released into wild populations. Explain your answer.

3 The fluorescent zebrafish in question has been the subject of heated debate both in Australia and overseas, and has been banned from sale in some countries. Form a group to discuss the ethical issues raised by this technology. Prepare a list of these issues.

4 Genetic technologies are increasingly more refined and applied to increasing numbers of organisms, often for commercial means. Canola is one such crop in Australia (Figure 3.1.26).

Figure 3.1.26 Canola crop

Enter keywords into a search engine to find out more about genetic transformation of canola.

a Suggest a reason that canola producers may favour genetically modified canola.

b Suggest a reason why consumers may be concerned about genetically modified food crops.

5 Write your own personal response to such applications of this technology in general and the fluorescent zebrafish in particular.

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A PCR simulation

Suggested duration: 60 minutes

INTRODUCTION

A significant quantity of DNA is needed for many DNA manipulations. When only small or even trace amounts are available, as may occur, for example, in a crime scene, polymerase chain reaction (PCR) can be applied to amplify that DNA. This makes millions of identical copies of the target DNA available for other applications such as gel electrophoresis and DNA profiling (Figure 3.1.31).

This investigation involves participating in a virtual laboratory activity. You will need to search the internet for a virtual PCR laboratory, such as the one available at the DNA Learning Center at Cold Spring Harbor Laboratory, USA.

AIM

- To simulate the process and outcome of polymerase chain reaction.
- To consider applications of polymerase chain reaction.

METHOD

Use key words to search for a virtual PCR laboratory. As a guide, the DNA Learning Center at Cold Spring Harbor Laboratory, USA provides a suitable example. Enter the site and read the information before launching the virtual laboratory. Follow the prompts to complete each step of the virtual PCR activity, answering the questions below as you proceed.

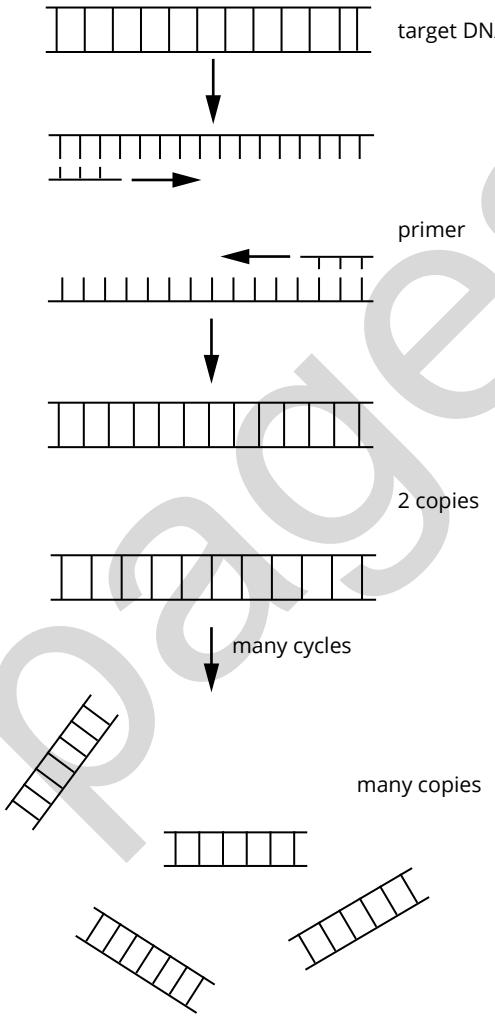

Figure 3.1.31 The polymerase chain reaction (PCR) process

1 Before undertaking PCR, DNA needs to be extracted from the nucleus of cells. List three possible sources of cells that could provide DNA for PCR.

2 What are primers? Describe their role in this reaction.

3 Name the four different nucleotide bases that have been added to the buffer solution. Why are they included?

4 What is meant by 'target DNA'?

5 Why has DNA polymerase been included in the buffer solution?

6 The DNA polymerase is not human polymerase, but rather *Taq* polymerase derived from the bacterium *Thermus aquaticus*, which inhabits hot springs. Outline the properties of *Taq* polymerase that make it a useful tool in PCR.

7 Describe what happens at the following temperatures.

a 95°C

b 50°C

c 72°C

8 How many copies of target DNA fragments are made after:

a three PCR cycles

b four PCR cycles

c five PCR cycles

d 30 PCR cycles

9 Describe the outcome of PCR.

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CONCLUSIONS

10 Summarise the process of PCR using a maximum of two sentences.

11 Suggest how the process of PCR is important to gene technologies such as gel electrophoresis and DNA profiling, which analyse DNA recovered at crime scenes and are used to investigate relationships between individuals.

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PRACTICAL ACTIVITY 4

Case study • Literature review

Genome editing using CRISPR-Cas9

Suggested duration: Part A—60 minutes; Part B—120 minutes

INTRODUCTION

CRISPR is an abbreviation for 'clustered regularly interspaced short palindromic repeats'. That means just what it says—short DNA sequences that are palindromic (read the same both forwards and backwards) and appear in clusters with regular spacing between them. The spacers contain unique DNA segments—that is, they are not repeats, but different from each other.

Why has CRISPR been the focus of so much attention in recent times if it is just DNA? And why is it so controversial?

CRISPR is a special kind of DNA found in bacteria, which use it to combat viral infections. In association with a protein called Cas9, scientists have been able to construct a bacterial CRISPR-Cas9 system that can edit DNA sequences in living cells. The possibilities for the application of this technology are profound. The genomes of organisms could be manipulated to confer desirable characteristics in plants and animals, for example to improve crop yield and quality in agriculture; to combat disease by altering bacteria and viruses; and to bypass genetically inherited diseases by correcting faulty genes.

In this activity you will find out more about CRISP-Cas9 and explore some possibilities the technology has to offer. You will consider some of the issues raised by the possible applications of CRISPR-Cas9 and make predictions about potential long-term consequences of some of its applications.

AIM

- To investigate the genome editing tool CRISPR-Cas9.
- To consider some issues related to the applications of genome editing.

BACKGROUND

The diagram below represents a simplified representation of the CRISPR-Cas9 molecule. Note the following features:

- the Cas9 enzyme
- a sequence of RNA called CRISPR RNA (crRNA)
- a sequence of RNA called tracer RNA (trcrRNA)
- a sequence of RNA called guide RNA (gRNA).

The diagram at the right of Figure 3.1.32 shows the crRNA joined to the trcrRNA. This combined RNA complex is referred to as the guide RNA (gRNA).

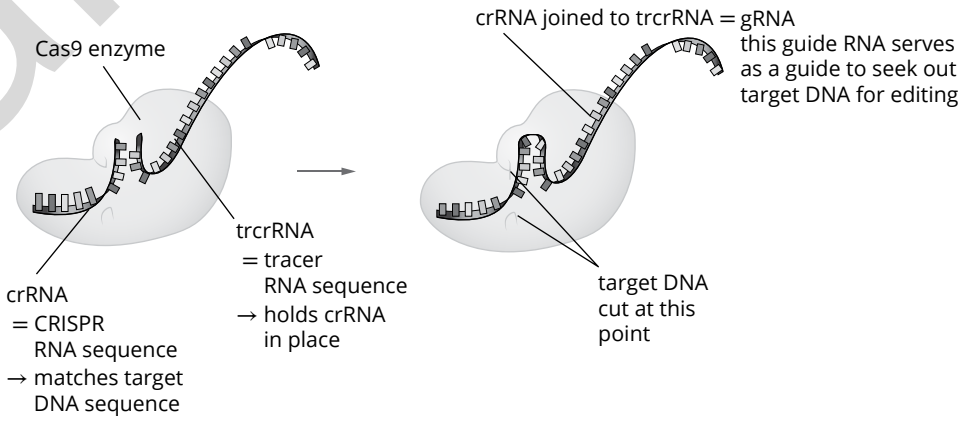

Figure 3.1.32 Guide RNA (gRNA)

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When a CRISPR-Cas9 system has been created to match a target sequence of DNA, it is ready to be introduced into affected cells for DNA editing. Figure 3.1.33 shows the CRISPR-Cas9 system working through the steps of editing a sequence of target DNA. DNA editing using the CRISPR-Cas9 system could include correcting a faulty gene by replacing a nucleotide, adding a DNA sequence or deleting a DNA sequence.

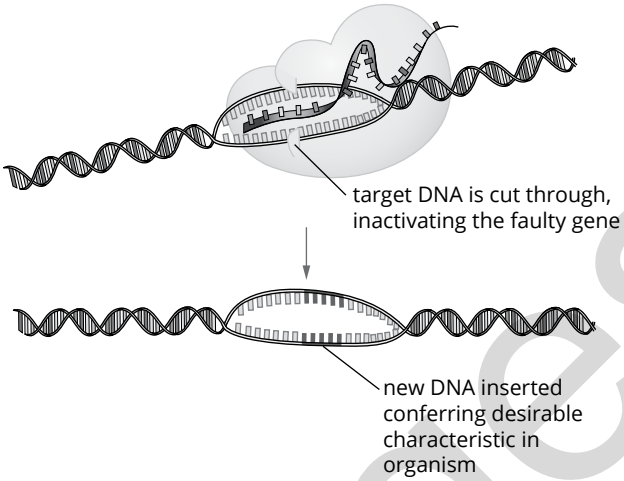

Figure 3.1.33 The CRISPR-Cas9 system editing a sequence of target DNA

Part A • CRISPR-Cas9 and its applications

Use the internet to locate and view a short animation video outlining the CRISPR-Cas9 system. Watch the video at least a couple of times, stopping and starting as needed. Useful key words/phrases for your search include CRISPR-Cas9 system; What is CRISPR?; explaining CRISPR.

1 Explain what is meant by each of the following terms.

a target DNA

b crRNA

c trcrRNA

d gRNA

2 In the CRISPR-Cas9 complex, describe the role of:

a the Cas9 enzyme

b guide RNA

3 **a** CRISPR-Cas9 technology has many potential applications. For example, it could be used to edit mosquito DNA as a means of controlling mosquito populations in malaria zones, with a view to eradicating malaria. Suggest other possible applications of CRISPR-Cas9.

b Share your response with other students, adding to your list of applications where relevant.

4 Outline how applying CRISP-Cas9 technology to edit somatic cells is different from applying it to edit germ cells.

5 Describe potential benefits from the development and application of CRISP-Cas9 as a genome-editing tool.

Part B • Issues around CRISPR-Cas9

The possibilities of CRISPR-Cas9 technology come hand-in-hand with some far-reaching implications and issues that are the subject of a great deal of controversy and ethical debate. Such issues fall under various contexts including social, cultural, economic, political and legal.

6 Suggest issues raised in the event that CRISPR-Cas9 technology were to be used as a tool in mosquito control to eradicate malaria.

7 Consider the potential applications of CRISP-Cas9 technology recorded in question 3a.

a Select one of these issues for further consideration. Identify the issue.

b Use resources to gather big picture information about the interplay between this potential CRISPR-Cas9 application in society in terms of:

- a social context
- a cultural context
- an economic context.

Include information about the following key points:

- Who are the stakeholders in the community?
- What are the perspectives held by the different stakeholders in terms of the technology and its outcomes?
- What arguments would they be likely to put forward to support their positions?
- How does the context influence the applications of the technology?
- What are the advantages and disadvantages of the technology in this application?
- What are the potential long-term outcomes?

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PRACTICAL ACTIVITY 4

Prepare a mind map summarising the information you have gathered.

8 A newspaper editorial is an article in which the editor takes in the information around an important issue affecting our society and outlines their opinion. Use all you have learned about your selected CRISPR-Cas9 application to write your own newspaper editorial on the application.

CONCLUSIONS

9 Use one or two sentences to summarise how the CRISPR-Cas9 system works to edit genes.

10 Write a short response to the information you have learned about CRISPR-Cas9 technology and its applications.

EXAM QUESTIONS

Multiple-choice questions

Question 1 VCE Biology 2016

In animal cells, tight junctions are multi-protein complexes that mediate cell-to-cell adhesion and regulate transport through the extracellular matrix. Proteins that form these complexes are made within the cell.

One pathway for the production of protein for these junctions is

A. nucleus—ribosome—Golgi apparatus—vesicle—endoplasmic reticulum.

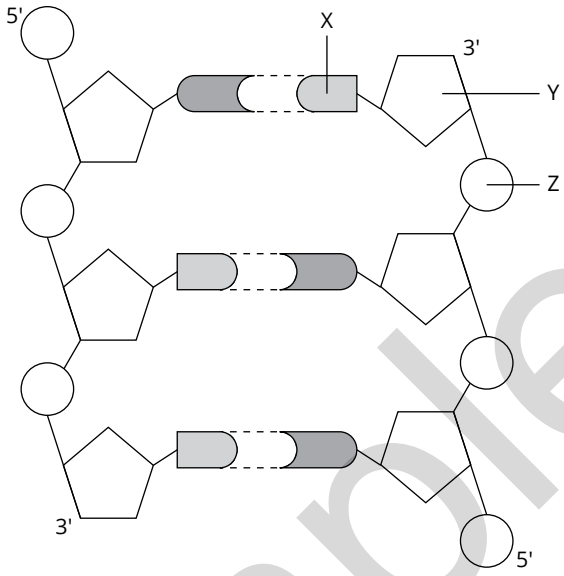
B. nucleus—ribosome—endoplasmic reticulum—vesicle—Golgi apparatus.

C. nucleus—vesicle—endoplasmic reticulum—Golgi apparatus—ribosome.

D. nucleus—vesicle—Golgi apparatus—ribosome—endoplasmic reticulum.

Use the following information to answer questions 2 and 3.

The diagram below represents part of a DNA molecule.



Question 2 VCE Biology 2015

A single DNA nucleotide is shown by sub-unit(s)

A. X alone.

B. X and Y together.

C. Y and Z together.

D. X, Y and Z together.

Question 3 VCE Biology 2015

A feature of DNA that can be seen in the diagram above is

A. the anti-parallel arrangement of the two strands of nucleotides.

B. the process of semi-conservative replication.

C. its ribose sugar–phosphate backbone.

D. its double-helix structure.

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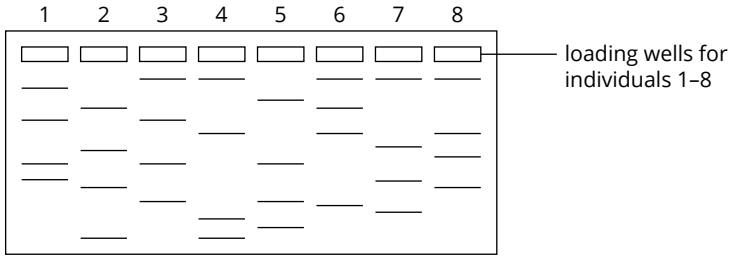
EXAM QUESTIONS

Question 9 VCE Biology 2018

Genetic testing can be used to test for the allele for Huntington's disease (HD). The onset of HD predominantly occurs in adulthood.

Eight individual family members were tested for the HD allele. The diagram below shows the electrophoresis gel results of a test for the presence of the allele. Individuals 4 and 8 have been diagnosed with the disease.



Which other individual is likely to suffer from HD now or in the future?

A. 1

B. 2

C. 5

D. 6

Question 10 VCE Biology 2017

The process known as polymerase chain reaction (PCR) involves repeated cycles made up of several steps.

During PCR the

A. first step in each cycle is to anneal primers to the DNA at a low temperature.

B. temperature must be lowered to 37°C before the beginning of each cycle.

C. second step in each cycle is to heat the DNA to a high temperature.

D. final step of each cycle involves the use of DNA polymerase.

Short-answer questions

Question 1 (6 marks) VCE Biology 2008

There are structural differences between molecules of DNA and RNA.

a. Outline two of these differences by completing the following table. 2 marks

	DNA	RNA
Difference 1		
Difference 2		

b. Name one kind of RNA and state its function. 1 mark

Type of RNA: _____

Function: _____

_____

EXAM QUESTIONS

Proteins may be classified as fibrous or globular depending on their three-dimensional shape.

In fibrous proteins, the polypeptide chains are arranged in parallel to form long fibres or sheets. In globular proteins, the polypeptide chains are folded into compact spherical or globular shapes.

c. Name the subunit of a polypeptide. 1 mark

Keratin, found in fingernails and claws, is an example of a fibrous protein.

d. Name another example of a fibrous protein and briefly outline its function. 1 mark

e. Describe a distinctive property of a fibrous protein and explain how this property is due to the arrangement of its polypeptides. 1 mark

Question 2 (6 marks) VCE Biology 2012 (1)

Human insulin is a macromolecule composed of two amino acid chains. The chains are connected by disulfide bonds.

a. To what group of macromolecules does insulin belong? 1 mark

Insulin found in other animals varies from human insulin.

The following table compares all the differences seen in the primary structure of human, cow, pig and sheep insulin.

	Amino acid position number within	
	Alpha chain	**Beta chain**
	-8 - 9 - 10-	**-30-**
human	-thr - ser - ile-	thr
cow	-ala - ser - val-	ala
pig	-thr - ser - ile-	ala
sheep	-ala - gly - val-	ala

b. What is meant by the term 'primary structure' of the insulin macromolecule? 1 mark

Humans with diabetes take insulin injections to maintain their health.

c. If supplies of human insulin were not available, which one of the other three animals listed in the table would be the best source of insulin? Explain your reason for choosing this particular animal. 2 marks

Animal: _____

Explanation: _____

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