Chapter 21
Biotechnology

Unit 3B

Answers to end of chapter questions

Please note that the following answers are sample answers only. There may be many alternative answers to the same question that are also correct. These are examples of correct answers.

Working scientifically

Activity 21.1 Restriction enzymes

Recombinant DNA technology or genetic engineering, as it is frequently called, involves the introduction into cells of fragments of DNA that are foreign to the organism. To do so, the strands of DNA under investigation need to be cut into useful fragments. The 'scissors' that cut the DNA are called restriction enzymes. The fragments can then be inserted into a suitable vector and joined with DNA ligase.

In this activity we will investigate how a sequence of DNA can be cut into suitable fragments using an appropriate restriction enzyme.

What to do

Answer the questions listed below. As you answer the questions refer to the relevant parts of this chapter where necessary.

1. Explain the following terms by describing their role in recombinant DNA technology:
(a) restriction enzymes

Answer
Restriction enzymes cut the DNA at sites that are identified by particular nucleotides. Some restriction enzymes produce a straight cut at the sequence (blunt ends), while others produce a staggered cut (sticky ends).

(b) recognition sites

Answer
The recognition site is the specific sequence of nucleotides in the DNA where the restriction enzyme cuts.

(c) blunt ends

Answer
A blunt end is that produced from a straight cut, which is when the restriction enzyme makes a clean break across the two strands of DNA. A blunt end is when both strands terminate in a base pair.

(d) sticky ends.

Answer
A sticky end is that produced from the staggered cut of specific restriction enzymes. Sticky ends, so called because of their ability to combine with sections of DNA that have a complementary ending, are very useful in recombinant DNA technology.

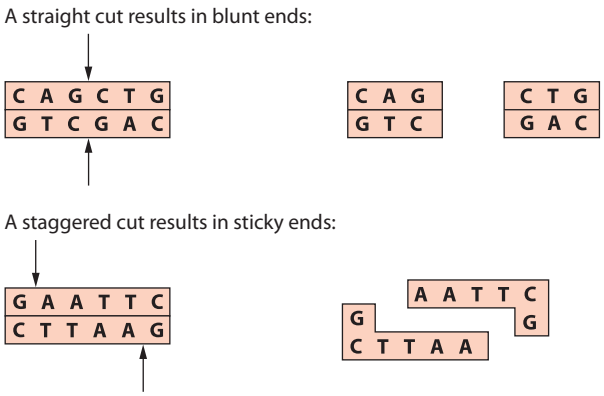

Figure 21.9 Cuts produced by restriction enzymes

2. Look at Figure 21.16 below and using Table 21.1 identify the restriction enzyme that is being used and the organism from which it was first isolated. What is the base sequence for this restriction enzyme's recognition site?

Answer
The restriction enzyme is Hind III from *Haemophilius influenzae.*

The recognition site is AAGCTT

TTCGAA

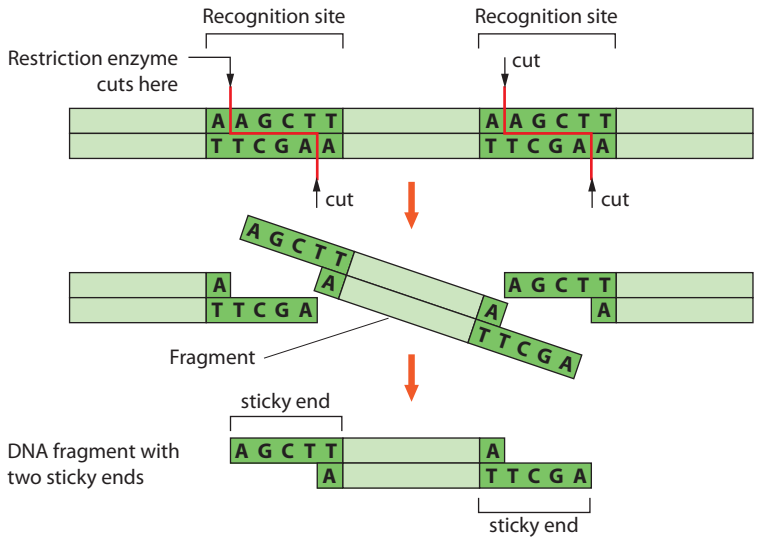

Figure 21.16 A restriction enzyme cuts a double-stranded DNA molecule at the recognition site

3. Imagine that you are a genetic engineer and need to cut the DNA sequence shown below. Using the four restriction enzymes listed in Table 21.2, study the sequence carefully and circle every recognition site that could be cut by each of the enzymes in turn. You may wish to use pens or pencils of four different colours.

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          10                  20                  30                  40                  50                  60
CATGGGTACG'CACAGTGGAT'CCACGTAGTA'TGCGATGCGT'AGTGTTTATG'GAGAGAAGAT'
          70                  80                  90                  100                 110                 120
CACGCGTCGC'CTTTTATCGA'TGCTGTACGG'ATGCGGAAGT'GGCGATGAGG'ATCCATGCAT'
          130                 140                 150                 160                 170                 180
ACGCGGCCGA'TCGAGTAATA'TATCGTGGCT'GCGTTTATTA'TCGTGACTAG'TAGCAGTATG'
          190                 200                 210                 220                 230                 240
CGATGTGACT' GATGCTATGC' TGACTATGCT'ATGTTTTTAT'GCTGGATCCA'GCGTAAGCAT'
          250                 260                 270                 280                 290                 300
ATCGCTGCGT'GGATCCCATA'TCCTTATATG'CATATATTCT'TATACGGATC'GAGCACGTTA'
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(a) Which of the enzymes produced the most fragments of DNA?

Answer
*Bam*HI

(b) Write down the recognition site for this enzyme.

Answer
GGATCC

(c) How many fragments of DNA have you created?

Answer
Five

(d) Were any of the other enzymes useful in cutting the strand of DNA? If so, how many fragments did it produce?

Answer
*Taq*I resulted in four fragments of DNA.

4. A process called ligation is used to reassemble the fragments. Name the enzyme involved in this process.

Answer
DNA ligase

5. Explain why the process of ligation can be viewed as the reverse of the restriction enzyme procedure.

Answer
Restriction enzymes cut DNA into fragments and DNA ligase joins DNA fragments.

6. In a short summarising statement explain why the discovery of restriction enzymes and DNA ligase has been so important for the advancement of genetic engineering.

Answer
Restriction enzymes and DNA ligase have allowed DNA recombinant technology to exist. By cutting and then rejoining strands of DNA genetic engineering has been able to advance. This technology has allowed researchers to identify some genetic disorders and develop treatments and even cures. For example the manufacture of vaccines and insulin and gene therapy.

Activity 21.2 Guest speaker

Genetics plays an important role in research into Huntington's disease. Gene therapy is a major source of hope for many Huntington's disease researchers. Other diseases such as

Parkinson's, Alzheimer's, cystic fibrosis, Duchenne muscular dystrophy and thalassaemia are all conditions that may be helped by innovations in biotechnology.

Most of these diseases have associations in each of the Australian states to help those with the disease and to provide support for their families. Most are also involved in educating the public about the effects of the particular disease and the progress being made in treatment. As a class, try to arrange for a speaker from one of these associations to present the latest information on research into the disease.

Activity 21.3 Investigating biotechnological techniques

Throughout this course in Human Biological Science you have had the opportunity to do many activities that have enabled you to work scientifically. This activity will allow you to apply some of those skills to investigate a particular biotechnological technique and create your own model of the process.

What to do

Working with a partner, or as part of a small group, select either polymerase chain reaction or DNA sequencing for further investigation. Both of these techniques have a number of stages that provide logical steps to allow models to be created.

1. Use a variety of references to establish the exact sequence of steps in the technique being investigated.
2. Draw a diagram to clearly illustrate all the steps in the process.
3. A scientific model is a simplified representation of an idea or a process. Using different shapes cut out of cardboard to represent the different parts of the process, build a simple model to demonstrate how the technique you are investigating takes place. Depending on the technique being investigated, your shapes may represent various nucleotides or segments of the DNA molecule. There will be many different ways of presenting the model so do not be surprised if yours is quite different from others.
4. Present and explain your model to the other members of the class.

Self-research

PCR

1. Denaturation
   DNA fragments heated, the DNA double helix splits into single strands.
2. Annealing
   The DNA is cooled down. Primers bind to the DNA template.
3. Extension
   DNA polymerase synthesises a complementary strand. Now a double helix again.
4. Repeat.

DNA sequencing

View animation at: http://www.wiley.com/college/pratt/0471393878/student/animations/dna_sequencing/index.html.

1. Polymerase chain reaction (PCR)
2. Sequencing reaction. The short pieces of DNA are used as a template to make many fragments all one base different in length.
3. Gel electrophoresis. These fragments are separated by gel electrophoresis

REVIEW QUESTIONS

1. Which groups of disease are now of significance to ageing Australians?

Answer
Cardiac and respiratory diseases, cancers, joint and muscle deterioration, neural diseases, especially Parkinson's and Alzheimer's diseases

2. (a) Why was the Human Genome Project set up?

Answer
The Human Genome Project became possible with advancements in technology. The project was set up to map the location of all the genes in the 46 human chromosomes.

(b) What have been some of the major outcomes to date?

Answer
Major outcomes of the Human Genome Project include:

- identification of specific genes involved in diseases (over 4000 identified so far)
- allowed gene replacement as a treatment for genetic diseases to become possible
- genetic test for hereditary diseases have been and are being developed
- monitoring gene expression and its relationship to the development of cancer.

(c) Give two examples of other lines of research that have benefited from the Human Genome Project.

Answer
Monitoring gene expression and its relationship to the development of colon cancer.

Genetic test for hereditary diseases have been and are being developed.

Pharmacogenetics—tailoring drugs to suit a person's genotype.

3. (a) What is a hereditary disease?

Answer
A hereditary disease is when a defective gene is passed from one generation to the next and thus is inherited.

(b) How are mutations associated with hereditary disease?

Answer
A mutation is when a gene produces a totally different characteristic instead of the one normally produced.

4. (a) What is DNA sequencing and what is it used for?

Answer
DNA sequencing determines the order of bases (nucleotides), and thus genes, in a sample of DNA.

(b) Briefly outline the steps in building a DNA sequence.

Answer
1. Each nucleotide is bonded to the previous one using the hydroxyl group.
2. A nucleotide without this hydroxyl group is bonded to the sequence.
3. This stops the DNA molecule from continuing to lengthen.
4. This sequence is then compared to the DNA sequence in question.

(c) How has DNA sequencing helped the treatment of hereditary spastic paraplegia?

Answer
People concerned that they may have hereditary spastic paraplegia can now undergo DNA screening before symptoms appear and start physical therapy to strengthen muscles.

(d) List other diseases for which DNA sequencing is proving a useful technique.

Answer
Sickle-cell anaemia, cystic fibrosis and some forms of cancer.

5. (a) Describe what is meant by the term 'DNA profile'.

Answer
To create a 'DNA profile' the sample of DNA is cut at particular base sequences and placed on a bed of gel. Electrophoresis results in the pieces of DNA forming a banding pattern dependent on the size of the DNA fragment. This banded picture is the person's DNA profile or fingerprint.

(b) List benefits that have arisen for those with hereditary disease from the use of DNA profiling.

Answer
Benefits are:

- used to identify if people carry the gene for cystic fibrosis or Huntington's disease
- used to identify alleles that my increase the risk of some conditions
- used for early identification for some hereditary disease so that early treatment can occur.

6. (a) Outline the steps in the polymerase chain reaction.

Answer
The steps in the polymerase chain reaction are:

- piece of DNA heated to 96°C to separate the two strands
- Primers attach to each strand of DNA
- new DNA strand synthesised from the primer using DNA polymerase
- this continues so that the original DNA sequence is replicated, then these two pieces of DNA replicated so there are four pieces and so on.

(b) Giving an example explain what is meant by the term 'heat stable DNA polymerase'?

Answer
Forms of the enzyme DNA polymerase that are heat stable do not denature or break down at high temperatures. One example is *Taq* polymerase from the heat loving bacterium, *Taq aquaticus.*

(c) List hereditary diseases for which treatment has improved due to the PCR technique.

Answer
Sickle-cell anaemia, PKU, cystic fibrosis and some viral diseases.

7. (a) What is a genetic probe and what are they used for?

Answer
A genetic probe is a piece of DNA or RNA that is radioactively labelled or labelled with fluorescent markers. It is used to detect particular base sequences in other DNA or RNA molecules.

(b) Which hereditary diseases have been detected using genetic probes?

Answer
Genetic probes have been used to detect Huntington's disease, cystic fibrosis, thalassemia and Duchenne muscular dystrophy.

8. What is recombinant DNA technology and what is the potential for the technique?

Answer
Recombinant DNA technology or genetic engineering uses foreign or altered DNA that is put into the body's cells and incorporated into the cell's DNA.

This technique has the potential to replace faulty genes, identify mutations and carriers of hereditary diseases.

9. Describe, with an example, what a transgenic organism is.

Answer
Transgenic organisms have one or more genes incorporated in their DNA from another organism, for example, a transgenic bacterium.

10. (a) What are restriction enzymes?

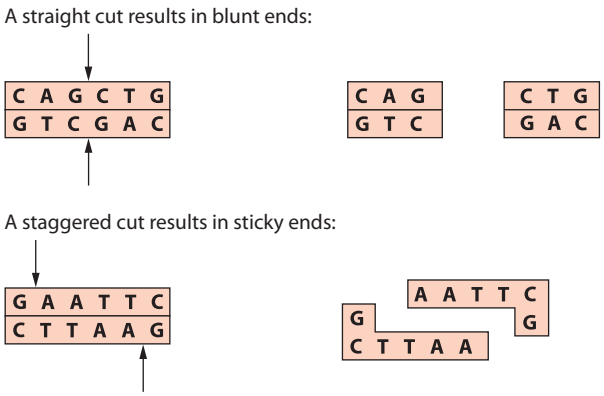
Answer
Restriction enzymes cut DNA at particular sequence of nucleotides and are so-named because they restrict the duplication of bacteriophages. They act as chemical scissors.

(b) List examples of restriction enzymes and for each, give their bacterial origin.

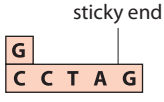
Enzyme	Bacterial origin
*Bam*HI	*Bacillus amyloliquefaciens*
*Eco*RI	*Escherichia coli*
*Hind*III	*Haemophilus influenzae*
*Taq*I	*Thermus acquaticus*

(c) Differentiate between 'sticky' and 'blunt' ends when used in relation to restriction enzymes.

Answer
Blunt ends: When DNA is cut, if both stands of DNA are cut with a matching base pair the end is blunt.



Sticky ends: When DNA is cut, if both stands of DNA are not cut with a matching base pair the end is sticky. There is one base that will 'stick' to another piece of DNA with the complimentary base.



11. What is DNA ligase and what is it used for?

Answer
DNA ligase joins fragments of DNA. It acts as a chemical glue and was originally called 'DNA-joining enzyme'.

12. (a) What are vectors and how are they utilised in recombinant DNA technology?

Answer
A vector is the means by which DNA is transferred from one cell to another, for example, by inserting the gene of interest into a bacterial plasmid or viral phage.

(b) List two different types of vectors that are used in this technology.

Answer
Bacterial plasmids and viral phages

13. How has treatment of the following diseases been assisted by recombinant DNA technology:
(a) diabetes;

Answer
Transgenic bacteria produce insulin that is collected and used by diabetics.

(b) human growth hormone; and

Answer
Transgenic *E. coli* produce human growth hormone that is collected.

(c) factor VIII.

Answer
Factor VIII is cultured in mammalian cells using recombinant DNA technology. It has the advantage of being a clean product and is free of plasma proteins that the recipient could be allergic to.

14. (a) What is gene therapy?

Answer
Gene therapy is the treatment of genetic disorders by replacing the faulty gene with a normal working gene.

(b) How is gene therapy likely to advance the treatment of cystic fibrosis and Huntington's disease?

Answer
Cystic fibrosis is caused by a single faulty gene. This gene could be replaced with a working gene before much or any damage occurs. This could alleviate symptoms or even cure the disease.

Huntington's disease is caused by a single gene. This gene could be silenced or shut off with gene therapy. This would alleviate symptoms or cure the patient.

15. (a) What is meant by the term 'cell replacement therapy'?

Answer
Cell replacement therapy replaces the cells of the human body that are damaged, not working properly or are missing.

(b) How will cell replacement therapy aid the treatment of diseases such as Parkinson's and Alzheimer's?

Answer
Neural crest stem cells, found in the hair follicles of adults, are (in some countries) used to grow new neural tissue that can replace dying tissue.

Embryonic stem cells can also be used to grow new neural tissue that can replace dying tissue.

Neural tissue that is transplanted can restore functioning of the nerves and reduce the symptoms of these diseases.

16. (a) What is the primary objective of tissue engineering?

Answer
To avoid organ transplants by restoring healthy tissue and organs.

(b) How are scaffolds used in tissue engineering? What are the qualities of a good scaffold?

Answer
Scaffolds are a template that cells are grown on so that they become a three-dimensional tissue.

Scaffolds should:

- have pore sizes to allow cell growth
- allow nutrient diffusion to the cells
- be biodegradable
- be able to be absorbed by the tissue
- allow tissue to absorb the scaffold at the same rate as the tissue growth.

(c) Give examples of materials used for creating scaffolds. Ensure you list both natural and synthetic materials in your answer.

Answer

Natural scaffolds	Synthetic scaffolds
collagen	polylactic acid
fibrin	

(d) List examples of tissues that can be produced by tissue engineering.

Answer
Bone, skin, adipose and cartilage

APPLY YOUR KNOWLEDGE

1. Widespread introduction of immunisation programs in Australia has led to an increase in life expectancy. Why are similar programs not effective for the elimination of cardiovascular and neural diseases?

Answer
Cardiovascular and neural disease can result from genetic disorders and/or dietary issues. These are not caused by a pathogen, thus the pathogen can not be used to produce a vaccine.

2. Population projections by the Australian Bureau of Statistics indicate that by the year 2051, the proportion of the total Australian population aged 65 years or more will almost double. Discuss how the impact of this shift in the age structure of the population will affect diseases of ageing such as Parkinson's and Alzheimer's, with particular reference to the stress it will create for health systems and resources.

Answer
With an increase in ageing population there would be an increase in the incidence of conditions that occur at this stage of life such as Parkinson's and Alzheimer's. This will mean that there will need to be extra care facilities available, including doctors, medications and nursing homes with suitably trained carers.

3. When the Human Genome Project was launched in 1990 it was expected to take until 2005 before complete mapping could be achieved. However, the results of the project were published in 2001, four years ahead of schedule. Find out what enabled the project to advance much faster than originally anticipated.

Answer
- Advances in computer technology
- International cooperation
- Advances in base sequencing
- Advances in technology.

4. The Human Genome Project is continuing to make information available about the human genome. Use an internet search engine to investigate the latest discoveries that have been made.

Answer
Some discoveries include:

- linking genes and sequences of DNA to particular diseases, including cancer, diabetes, blindness and AIDS
- linking genes and sequences of DNA that predispose someone to having a heart attack
- discovery of new medications and drugs
- identification of genetic variations that contribute to the risk of having diabetes, Parkinson's disease, heart disorders, obesity, Crohn's disease and prostate cancer.

See http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml.

5. How do mutant genes contribute to disease? Discuss the techniques that are available to detect the presence of a mutant gene.

Answer
Mutant genes can cause or contribute to disease. The gene that has mutated may not work properly or not work at all and this can lead or contribute to diseases. These mutant genes can be detected by genetic testing. These tests can include sequencing of the suspected gene and the use of DNA probes.

6. One of the most frequently used ways to sequence DNA is to take advantage of the way DNA replicates. Explain how it is that if the sequence of bases on one side of a fragment of DNA is known, the sequence on the other side is known as well.

Answer
DNA is a double-stranded molecule that has complementary base pairs. So if one strands base sequence is known then the other strands sequence will be the

complementary bases. Adenine pairs with thymine so wherever adenine appears on one half of the DNA molecule, thymine will be on the other half and vice versa. Cytosine pairs with guanine so wherever cytosine appears on one half of the DNA molecule, guanine will be on the other half and vice versa.

7. In this chapter, DNA sequencing was discussed in relation to hereditary spastic paraplegia. Find out how DNA sequencing is being used with other diseases such as sickle-cell anaemia.

Answer
DNA sequencing was used to determine the actual mutation that causes sickle-cell anaemia. It has been found that the mutant gene is the HBB gene located on the short arm of chromosome 11. The mutation involves the change in just one base pair in the DNA sequence within the gene. The DNA sequencing for sickle-cell anaemia is being used to look at the proteins encoded for in producing both foetal and adult haemoglobin. It is also being used to see how the proteins turn on the foetal haemoglobin in adults as a treatment for sickle-cell anaemia.

8. With DNA profiling, genetically inherited diseases can be detected at an early age. Discuss the advantages of the early detection of a particular genetic disease.

Answer
Generally early detection allows preventative therapies, strengthening of the individual, medical procedures, reduction in symptoms and management of the disease. The aim is to have early detection and eventually a cure before symptoms appear. Early detection may also help couples to assess the implications of having children.

9. The polymerase chain reaction is a method of amplifying a small amount of DNA into a much larger amount. What are the advantages of being able to do this, and how is it helpful in the detection of hereditary diseases?

Answer
The advantages are:

- decreased amount of time to detect hereditary disease
- the targeting of the specific gene that causes hereditary disease (i.e. do not need to sequence all DNA)
- the use of DNA from small specimen, for example, a drop of blood or a strand of hair.

10. The use of blood products sourced from living donors and human growth hormone from cadavers resulted in products that were devised to improve the quality of life but which also had life-threatening side effects. Use an internet search engine to find out the types of diseases that were involved with these contaminated products and how they affected the recipients of those products. How has recombinant DNA technology overcome these life-threatening side effects?

Answer
Diseases involved:

- disease from contaminated human growth hormone
- Creutzfeldt-Jakob disease (caused degeneration of the brain and was fatal)
- HIV/AIDS, hepatitis and some other blood-borne diseases.

Recombinant DNA technology means that the production of human growth hormone is clean. Only the hormone is produced. No other proteins or cell products that cause negative side effects are made. Blood clotting factors are now produced using recombinant DNA technology.

11. One researcher in the United States stated: 'Tissue engineering holds out promise of truly healing the heart after congestive heart failure. Through tissue engineering we could actually restore the function of the heart by replacing large portions of the damaged heart muscle by a bioartificial one.' This same researcher has been working for a long time on developing the ideal scaffolding to support the injected cells and the architecture of the heart. Use an internet search engine to find out the type of scaffolding material that is being used in such research and the success that has been achieved to date.

Answer

A honeycomb polymer scaffold that stretches like cardiac muscle, passes electrical impulse more in one direction than the other, and guides the cultured cells to grow on the scaffold. This is still in the research stages using rat cells.

12. In January 2009 it was announced that a woman in Britain gave birth to a baby that had grown from an embryo that had been genetically screened to ensure it was free of the BRCA 1 gene. Any girl born with this gene has an 80% risk of developing breast cancer, and the mother was particularly concerned as several of her husband's close female relatives had developed the disease. Discuss the risks and ethical concerns relating to such genetic screening.

Answer

The risks are:

- damage to the embryo during cell removal
- single cell testing has problems—may be a one-off cell
- testing may not be accurate
- increased risk of miscarriage with increasing maternal age
- reduced pregnancy rate (implantation) after screening.

The ethical concerns are:

- designer baby not natural/playing God
- designer baby not God's intent
- reduced variation in population
- develop a super race—affect social attitudes.

13. Biotechnology is increasingly impacting on our daily lives. Much is being said and written about developments in the use of stem cells to aid the treatment of disease. Have a class debate so that both sides of the question, 'Should the Australian federal government support embryonic stem cell research?' can be canvassed. Remember to keep an open mind and respect the opinions of others.

Answer

Yes	No
• We have the technology, why not use it? • Advances in medicine • Potential for cures and treatment of those in pain and suffering	• Playing God • Kills embryos • Not enough testing and evidence to say it is a success • Is it really safe? • Embryonic stem cells could differentiate into cancers • Research driven by profit and scientific advancement not cures