SUGGESTED TEXTBOOK ANSWERS

Chapter 13 Techniques in biotechnology

The following are suggested answers only. Other answers to the same questions may also be correct.

Science inquiry

Activity 13.1 Electrophoresis simulation

1 What ingredients are used to make the gel?

Answer: The gel ingredients consist of powdered agarose (agarose is one of the main constituents of agar) and buffer (a salt solution).

2 Describe how the gel is made using the ingredients that you have listed.

Answer: Powdered agarose and buffer solution are mixed together in a flask and then heated (in a microwave) so that the agarose melts into the buffer solution.

3 DNA samples are placed in wells in the gel. Explain how the wells are made.

Answer: A device like a comb is placed into the melted solution. It is left in place until the gel solidifies. When the comb is removed wells are left in the gel.

4 What is the purpose of the DNA size standard?

Answer: The DNA size standard contains DNA fragments of known length which can then be used as reference points by which the length of the unknown DNA fragments can be estimated.

5 What electrical charge does a DNA molecule have?

Answer: DNA has a negative charge.

6 Which electrical charge is applied to the well end of the gel?

Answer: The negative charge

7 Is it possible to tell whether an electric current is running through the gel?

Answer: When the current is running, air bubbles appear on the electrodes at each end of the electrophoresis box.

8 What makes the DNA migrate through the gel?

Answer: The negatively charged DNA fragments are repelled by the negative charge at the well end of the electrophoresis box.

9 Describe the technique that is used to make the DNA visible in the gel.

Answer: The gel is taken out of its mould and placed in a solution that stains the DNA so that it can be seen.

10 Why do shorter DNA strands move further through the gel than longer strands?

Answer: The gel acts as a filter and smaller molecules move through the filter more rapidly than larger molecules.

Activity 13.2 Restriction enzymes

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.

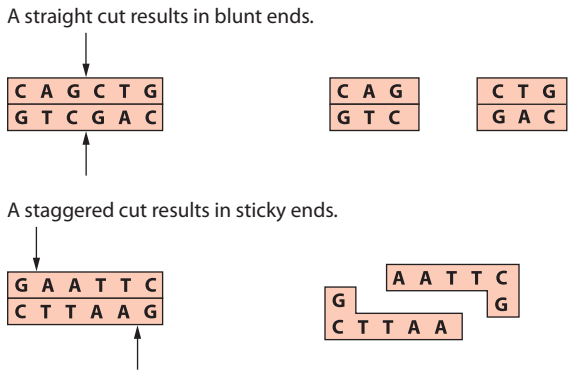

Figure 13.7 Cuts produced by restriction enzymes

2 Look at Figure 13.12 and, using Table 13.1 (on page 183), 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:

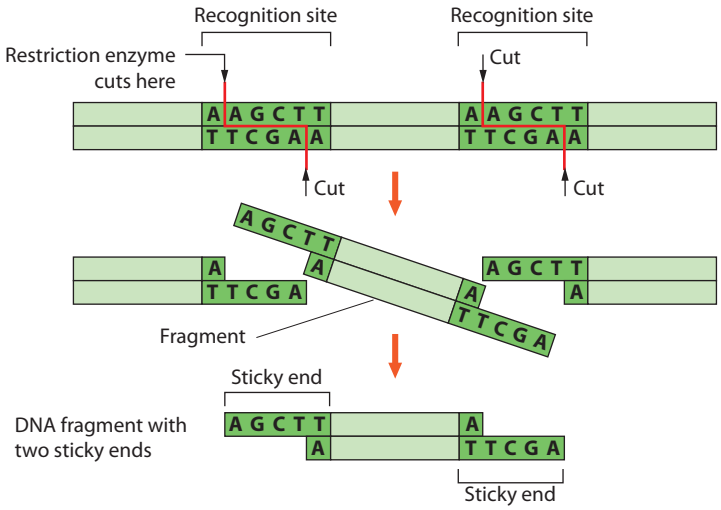

Figure 13.12 A restriction enzyme cuts a double-stranded DNA molecule at the recognition site

The restriction enzyme is *Hind*III from *Haemophilus influenzae.*

The recognition site is $\frac{\text{AAGCTT}}{\text{TTCGAA}}$

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 13.1 (page 183), 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.

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'

a Which of the enzymes produced the most fragments of DNA?

Answer: *Bam*HI shaded grey above.

b Write down the recognition site for this enzyme.

Answer: GGATCC

c How many fragments of DNA have you created?

Answer: Five

d Was any other enzyme useful in cutting the strand of DNA? If so, how many fragments did it produce?

Answer: *Taq*I (shaded pink above) 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 develop. By cutting, and then rejoining strands of DNA, genetic engineering has been able to advance. For example, the manufacture of vaccines and insulin has been accomplished using recombinant DNA technology. Gene therapy is likely to become possible on an increasing scale.

Activity 13.3 Investigating biotechnological techniques

4 Present and explain your model to the other members of the class.

Answer: For the polymerase chain reaction, students should show:

- Denaturation: DNA fragments heated, the DNA double helix splits into single strands.
- The DNA is cooled down. Primers bind to complementary places on the DNA strands (this process may be called annealing).
- DNA polymerase brings about synthesis of a complementary strand of DNA so that a double helix is formed again.
- The process repeats over and over again, each time doubling the number of DNA molecules produced.

For DNA sequencing, students should show:

- Polymerase chain reaction (PCR) may be carried out to increase the amount of DNA for profiling.
- Enzymes are used to cut the DNA into short lengths. The cuts take place at specific points.
- The short pieces of DNA are used as a template to make many fragments.
- These fragments are separated by gel electrophoresis – the fragments migrate through a gel that is electrically charged; smaller fragments move faster than larger ones.

Review questions

1 a Why was the Human Genome Project set up?

Answer: The Human Genome 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 genetic disorders identified so far)
- gene replacement as a treatment for genetic diseases becoming possible
- development of genetic tests for hereditary diseases
- monitoring gene expression and its relationship to the development of cancers.

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 testing for some hereditary diseases is now possible and many more will be developed.

Pharmacogenetics – tailoring drugs to suit a person's genotype will become increasingly common.

2 a What is DNA sequencing and what is it used for?

Answer: DNA sequencing is the process of determining the order of bases (nucleotides), and thus genes, in a sample of DNA.

b Briefly outline the steps in building a DNA sequence.

Answer: Steps involved in building a DNA sequence include the following:

- DNA is extracted from cell nuclei.
- DNA is broken into pieces and the pieces copied many times.
- Double stranded DNA separated into single strands.
- Primer added and binds to one strand.
- The second DNA strand is recreated by adding nucleotides in the correct sequence.
- A terminator nucleotide stops the nucleotide sequence.
- Strands can then be compared to determine the nucleotide sequence.

c Name three diseases for which DNA sequencing is a useful technique.

Answer: Such diseases include:

- spastic paraplegia
- sickle-cell anaemia
- cystic fibrosis
- some forms of cancer.

3 a What is a 'DNA profile'?

Answer: A sample of a person's 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 two practical applications of DNA profiling.

Answer: DNA profiling can be used:

- in forensic science to match DNA found at a crime scene with that of a suspect
- in tracing ancestry to show relationships between related people
- to identify genes that cause inherited disease – often enabling early diagnosis of disease
- in families where there is a history of inherited disease to determine the chances of having a child with the disease.

4 a Outline the steps in the polymerase chain reaction.

Answer: The steps in the polymerase chain reaction include the following:

- DNA is heated to 96°C to separate the two strands of the molecule (denaturation).
- Primers are added and attach to each strand.
- Cooling occurs so that the new DNA strand is synthesised when the primer initiates the reaction with DNA polymerase.
- Heating occurs to separate the strands of the new DNA molecules and the process is repeated.

b Giving an example, explain what the term 'heat stable DNA polymerase' means.

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

c What are some of the practical applications of the polymerase chain reaction?

Answer: The polymerase chain reaction can be used to:

- reduce the time taken to identify hereditary disease in a person's genome
- detect viral infections, often before symptoms of disease occur
- amplify DNA from small samples left at crime scenes
- compare genomes of fossils where the fossils contain small amounts of DNA that can be extracted.

5 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 cells of another species of organism and becomes incorporated into the cell's DNA.

This technique has the potential to replace faulty genes. It has also been used to place genes into organisms like yeasts or bacteria so that the organisms produce pure proteins for medical products such as insulin and growth hormone.

6 Explain, with an example, what a transgenic organism is.

Answer: Transgenic organisms have one or more genes incorporated into their DNA from another organism. For example, transgenic bacteria have been genetically engineered to produce useful proteins such as insulin.

7 a What are restriction enzymes?

Answer: Restriction enzymes cut DNA at particular sequence of nucleotides. They act as chemical scissors. (The name restriction enzymes was used because they restrict the duplication of bacteriophages, viruses that infect bacterial cells.)

b List examples of restriction enzymes. For each, give their bacterial origin.

Answer:

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 in relation to restriction enzymes.

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

Sticky ends: When DNA is cut, the two strands of the DNA molecule are not cut with a matching base pair. This means that the end is uneven or 'sticky'. There is one base that will 'stick' to another piece of DNA with the complementary base.

See also the Figure 13.7 on page 183.

8 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'. It is used to splice genes (groups of nucleotides) into a DNA molecule.

9 a What are vectors and how are they used in recombinant DNA technology?

Answer: A vector is the means by which part of a DNA molecule is transferred from the cell of one organism into a cell of another organism. For example, by inserting the gene of interest into a bacterial plasmid or viral phage, which will then act as a vector to transfer the gene to another cell.

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

Answer: Bacterial plasmids and viral phages.

10 How has treatment of the following diseases been assisted by recombinant DNA technology?

a Diabetes

Answer: Insulin used to be extracted from the pancreas of cattle and pigs. Now transgenic bacteria are used to produce insulin that is collected and used by diabetics. The insulin produced by the transgenic bacteria is identical to human insulin and does not produce the side effects that were experienced by some patients when insulin from cattle and pigs was used.

b Deficiency of human growth hormone

Answer: Human growth hormone used to be extracted from human bodies to treat patients who were deficient in the hormone. Supply was a problem and there was also a risk of transmission of disease. Now transgenic *E. coli* produce human growth hormone.

c Haemophilia A

Answer: Patients suffering from haemophilia A are deficient in a blood clotting protein called factor VIII. This clotting factor used to be extracted from the plasma of donated blood so there were problems getting enough to treat haemophiliacs and there was also a risk of transmission of blood borne diseases. It is now cultured in mammalian cells using recombinant DNA technology. It has the added advantage of being a clean product and free of plasma proteins to which the recipient could be allergic.

11 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 changes its structure and produces a totally different characteristic instead of the one normally produced. The new characteristic may manifest itself as a disease. This may then be passed on to the next generation if the mutation occurs in a sex cell.

12 a What is gene therapy?

Answer: Gene therapy is the treatment of genetic disorders by replacing the faulty gene with a gene that works normally.

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. Using gene therapy, this gene could be replaced with a normal 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.

13 a Define 'cell replacement therapy'.

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

b How could 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.

Transplanted neural tissue could restore functioning of nerves and reduce the symptoms of Alzheimer's, Parkinson's and other diseases.

14 a What is the primary objective of tissue engineering?

Answer: Tissue engineering can be used to avoid organ transplants or implants of synthetic materials by restoring healthy tissue and organs.

b How are scaffolds used in tissue engineering?

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

- have pore sizes that 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.

Apply your knowledge

1 When the Human Genome Project was launched in 1990 it was expected to take until 2005 for complete mapping to 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: The project proceeded faster than expected because of:

- international cooperation
- automation of the polymerase chain reaction (allowing rapid sequencing of bases)
- advances in computing technology.

2 The Human Genome Project is continuing to make information available about the human genome. Use an Internet search engine to investigate the latest discoveries.

Answer: Some discoveries include:

- linking genes and sequences of DNA to particular diseases, including cancer, diabetes, some forms of 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 also www.ornl.gov/sci/techresources/Human_Genome/project/benefits.shtml.

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

Answer: A mutant gene may not work properly or not work at all and this can lead to disease. These mutant genes can be detected by genetic testing. These tests can include sequencing of the suspected gene and the use of DNA probes.

4 One of the most frequently used ways to sequence DNA is to take advantage of the way DNA replicates. Explain how, 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 strand's base sequence is known then the other strand's sequence will be the complementary bases. Adenine pairs with thymine so wherever adenine appears on one strand of the DNA molecule, thymine will be on the other strand 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.

5 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: Early detection may allow preventative therapies to be used or medical procedures that reduce symptoms and allow the disease to be managed. The aim is to have early detection and eventually a cure before symptoms appear. Early detection may also help couples to decide whether to terminate a pregnancy

6 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?

Answer: The advantages are:

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

7 The use of blood products sourced from living donors and human growth hormone from cadavers resulted in products that were devised to improve quality of life but which also had life-threatening side effects. Using the Internet, 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: The use of blood products from living donors carried the risk of diseases such as hepatitis B and HIV. The use of recombinant DNA to produce blood clotting factors has eliminated this risk.

The main threat from cadaver growth hormone was the transmission of Creutzfeldt-Jakob disease, a form of mad cow disease. By 2003 the numbers of patients treated with cadaver growth hormone who had contracted CJD was so high that use of the hormone from cadavers was stopped. Recombinant DNA technology means that production of human growth hormone is clean. Only the hormone is produced. No other proteins or cell products that could cause negative side effects are made.

8 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 impulses 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. See:
http://web.mit.edu/newsoffice/2008/heart-1102.html
http://www.nature.com/news/tissue-engineering-how-to-build-a-heart-1.13327.

9 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 BRCA1 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 (in the case of pre-implantation genetic testing)
- testing may not be accurate
- increased risk of miscarriage with increasing maternal age
- pre-implantation testing may interfere with later implantation.

The ethical concerns are:

- designer babies not natural (the practice is 'playing God')
- tests may identify problem genes, but cannot predict how severely the baby will be affected
- some genetic disorders only manifest themselves after exposure to certain environmental conditions
- reduced variation in population
- parent's may decide on termination for non-medical reasons; for example, to produce a child of a particular sex.

10 The impact of biotechnology on our daily lives is growing. Much is being said and written about developments in the use of stem cells to aid the treatment of disease. Hold a class debate to canvas both sides of the question: 'Should the Australian federal government support embryonic stem cell research?' Remember to keep an open mind and respect the opinions of others.

Answer:

Yes	**No**
We have the technology, why not use it?	The embryos, which have the potential to develop into human beings, are killed in the research
Advances in medicine could benefit everyone	Chances of success are doubtful
Potential for cures and treatment of those in pain and suffering	Is it really safe?
	Embryonic stem cells could differentiate into cancers
	Research may be driven by profit and scientific advancement, not cures

11 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. There will be a need for extra care facilities, including doctors, medications and nursing homes with suitably trained carers. This in turn will create problems for the country's economy because of the cost of providing such facilities.