This chapter is designed to enable students to:

- recognise that DNA can be manipulated in various ways, such as by cutting, joining, and copying
- identify various techniques used to manipulate DNA
- distinguish between endonucleases and exonucleases
- become familiar with the role of the polymerase chain reaction in producing copies of DNA
- develop awareness of the use of plasmids as vectors to transform bacterial cells.
A recipe for gene editing

The first attempts at genetic manipulation in the 1970s involved adding DNA to the plant and animal genomes.

Early techniques used to add DNA to target cells included:
- the use of so-called ‘gene guns’ in which gold atoms coated with DNA were blasted into cells, a technique used mainly to insert genes for pesticide or herbicide resistance into plant cells
- the use of modified viruses to act as vectors to carry a functional gene into cells with a defective gene. Viruses used as vectors include retroviruses and adenoviruses.

These techniques were clearly very limited because there was no control over where any added DNA would be inserted into the genome of the target cells. Instead, DNA was simply added randomly into the genome. What would be expected to happen if the added DNA was by chance inserted into a gene that was essential for cell survival, causing the gene to become non-functional? What would be expected to happen if the added DNA was by chance inserted into a cancer-suppressing gene so that this gene was disabled?

Another major limitation was that, although these early techniques could carry a functional replacement copy of a gene into cells with a defective gene, these techniques were not able to mend a defective gene by editing or disabling it. Editing is a process of correction, such as occurs when an editor of a manuscript corrects a misspelt word, or adds or replaces a word or phrase, or deletes a sentence.

What was missing was an efficient and reliable technique for making precise and targeted changes to the genome of living cells. Such a process is called gene editing or genome editing or DNA editing. Gene editing refers to changing a genomic DNA sequence in some way. This might involve making a single base change to a gene, such as a base substitution, addition or deletion. Gene editing on a larger scale might involve disabling, replacing or adding a gene, or changing an upstream DNA sequence that regulates a gene. However, in 2012, a ‘game-changing’ discovery was made.

In 2012, an article in the journal Science (Jinek, M. et al. Science 337, 816–821) described a gene-editing technique that is called CRISPR-Cas9 (often just shortened to CRISPR and pronounced ‘crisper’). Interestingly, CRISPR is part of the adaptive immune system of bacteria that chops any DNA of invading viruses, and has been operating in the microbial world for many thousands of millions of years.

What was most significant was the recognition that CRISPR-Cas9 technology could be readily adapted to provide an inexpensive and easy-to-use means of genome editing for use in an endless range of genes from any organism. The co-inventors of this exciting gene-editing technique were two women scientists, Dr Jennifer Doudna (refer back to figure 13.1) and Dr Emmanuelle Charpentier (see figure 13.2).

This tool, borrowed and adapted from the bacterial adaptive immune system, is now being put to use in editing faulty genes and in silencing genes in plants and animals. Many applications in agriculture are being explored (see chapter 15, page 706). Research is already underway on the
use of the CRISPR technology as a safe and reliable means of editing the defective alleles responsible for human diseases, such as cystic fibrosis and sickle-cell anaemia.

**How does CRISPR-Cas9 work?**

The CRISPR gene-editing technique simply requires molecular ‘scissors’ to cut the target DNA and a ‘guide’ to direct the ‘scissors’ to the site where the cuts in the DNA will be made.

These two tools are as follows:

1. The ‘scissors’ are Cas9 nuclease, a bacterial enzyme that:
   (i) can unwind double-stranded DNA
   (ii) can cut both strands of double-stranded DNA at a precise location.
2. The ‘guide’ is a segment of an artificially synthesised single strand of RNA. This guide RNA is designed to include a 20-base sequence that is complementary to part of the target DNA.

What gives the CRISPR-Cas9 technology its potential remarkable range of use?

Firstly, guide RNA molecules can be designed with a 20-base sequence that can home in on any segment of genomic DNA from any organism. Secondly, the Cas9 enzyme can cut any double-stranded DNA. This is a reminder of the universality of DNA as the material of inheritance.

How does the CRISPR technology work?

The cell containing the DNA to be edited is transfected with the Cas9 nuclease enzyme and the guide RNA. The guide RNA hybridises with its complementary DNA sequence and this identifies the position where the Cas9 nuclease enzyme will cut the DNA. The cut is made by the Cas9 nuclease at a location just upstream from a specific three-base sequence called PAM.

![Stylised diagram showing the components of CRISPR-Cas9 technology](image)

**Figure 13.3** Stylised diagram showing the components of CRISPR-Cas9 technology. Note the single-stranded guide RNA (sgRNA) with its leading segment (shown in orange) that is complementary to the DNA of interest. The sgRNA enables the Cas9 nuclease enzyme (shown as the large blue shape) to be positioned to cut both strands of the DNA close to the PAM site (shown in red). The two pairs of scissors indicate the DNA sites cut by the Cas9 nuclease.
After the Cas9 nuclease enzyme has cut both strands of the DNA of interest, the breaks can be repaired and, in this process, two kinds of gene editing can occur.

1. In one type of repair process, called a gene ‘knock in,’ a specially designed DNA sequence is inserted into a precise location in the genome (see figure 13.4). The aim of this type of repair is to edit faulty alleles and restore their normal function. For example, using this process, the gene mutation responsible for cystic fibrosis has been corrected in cultured stem cells taken from individuals with cystic fibrosis.

2. In the second type of repair, called a gene ‘knock out,’ the re-joining that repairs the break in the DNA involves a process that is subject to error. This process can result in a random insertion or deletion (indel) of one or two bases, producing a frameshift mutation. A frameshift mutation can either disable a gene or can produce a STOP signal. So, the aim of this type of repair is to disable or silence a gene (see figure 13.5).

**What about the future?**

CRISPR-Cas9 technology has extraordinary potential for widespread use in clinical, agricultural, and research settings, for example:

- for a myriad of research purposes:
  - to ‘knock out’ genes, one at a time, in order to identify their function
  - to introduce specific mutations in a DNA sequence.
- to edit a faulty allele of a gene in a person with a severe inherited disease
- to snip out the faulty segment of a gene and replace it with a working copy
- to activate or to repress a gene
- to add a new gene to the genome.

This potential is highlighted by the fact that major pharmaceutical companies have made significant investments in CRISPR-Cas9 technologies. For example, in October 2015, Vertex Pharmaceuticals paid in excess of US$100 million to use the gene-editing technology of CRISPR Therapeutics. Their intention is to develop treatments for cystic fibrosis and sickle-cell diseases. More recently, Bayer Pharmaceuticals and CRISPR Therapeutics set up a joint venture aimed at developing therapies to treat blood disorders, blindness and congenital heart disease.
In a special review in the international journal *Nature* (25 May 2015), CRISPR technology was introduced as follows:

**CRISPR: THE GOOD, THE BAD AND THE UNKNOWN**

‘A DNA-editing technology called CRISPR has rapidly become one of the most popular ways to alter genomes. Concerns about its risks temper excitement about its usefulness. It has already been used to modify human embryos, and the technology could alter wild animal populations; it works in everything from wheat to mice…’

CRISPR-Cas9 technology itself is neutral — it is a cut and paste or cut and replace technique that can operate on the genomic DNA of a human cell or a mouse cell or a mosquito cell or a wheat cell or a yeast cell, and the list goes on…

What about the good, the bad and the unknown? Like any technology, CRISPR will be identified as good or bad depending on the uses to which it is put and their immediate and longer term consequences. As the technology is applied more widely, more unknowns will emerge.

In April 2015, a group of Chinese scientists reported the results of their use of CRISPR technology on human embryos in an attempt to edit a faulty gene. (The embryos came from an in-vitro clinic and, because each was the product of fertilisation by two sperm, these embryos were not capable of completing development.) The results of their experiments appear to have raised many questions and few answers.

Issues being debated include: Should gene editing be allowed on human cells, such as eggs, sperm and embryos, whose DNA can pass to future generations? Or, should gene editing be restricted to somatic cells where that DNA is not passed to the next generation? These and related ethical issues will continue to engage scientific communities, governments and the public.

In late 2015, the first International Summit on Human Gene Editing was held in the United States in Washington, DC. The following headline from a Canadian newspaper that reported on this meeting encapsulates the situation: **CRISPR gene-editing tool has scientists thrilled — but nervous**. (Source of headline: Crowe, Kelly 2015 ‘CRISPR gene-editing tool has scientists thrilled — but nervous’; CBC News, 30 November.)

CRISPR-Cas9 is one technique that involves the manipulation of DNA at the level of genes or even the genome. In the following sections, we will explore some of the other tools that can be used to manipulate DNA.

**KEY IDEAS**

- CRISPR-Cas9 technology is an efficient, easy-to-use, and inexpensive tool for precise gene editing.
- CRISPR-Cas9 technology can be applied to every eukaryotic species, including humans.
- The key components of CRISPR-Cas9 technology are a guide RNA and an endonuclease enzyme, Cas9.
- Early gene therapy techniques added DNA segments to genomes but at unpredictable locations.
- CRISPR-Cas9 technology enables precise genomic editing.
- CRISPR technology can not only add DNA but can also edit, modify or disable or delete DNA from the genome.
- Like any new technology with so many potential applications, aspects of the use of CRISPR technology raise ethical considerations.
Tools to manipulate DNA

Carpenters work with wood and sculptors work with clay. Scientists who use gene manipulation technology are sometimes called ‘genetic engineers.’ Genetic engineers work with the genetic material DNA.

Each operator — carpenter, sculptor or genetic engineer — requires tools to manipulate the raw material. To construct a bookcase, a carpenter needs tools such as a ruler, saw and hammer so that the wood may be measured, cut and joined. Similarly, the genetic engineer requires tools to cut, join, copy and separate DNA. The following section describes the tools and techniques that, when combined, enable genes to be manipulated in various ways.

Table 13.1 shows some of the ‘tools’ used in gene manipulation. The same tools can be used regardless of the source of the genetic material.

### Table 13.1 Tools for gene manipulation.

<table>
<thead>
<tr>
<th>Action</th>
<th>‘Tool’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut DNA into fragments at precise locations</td>
<td>restriction enzymes (see Cutting DNA into fragments)</td>
</tr>
<tr>
<td>Separate fragments by size</td>
<td>electrophoresis (see Sorting DNA fragments)</td>
</tr>
<tr>
<td>Find particular DNA fragments</td>
<td>probes (see Finding DNA fragments of interest)</td>
</tr>
<tr>
<td>Join DNA fragments</td>
<td>ligase enzyme (see Joining DNA fragments)</td>
</tr>
<tr>
<td>Make specific DNA fragments</td>
<td>DNA synthesiser &amp; reverse transcriptase (see Making DNA)</td>
</tr>
<tr>
<td>Amplify DNA</td>
<td>Polymerase chain reaction (PCR) (see Amplifying DNA)</td>
</tr>
</tbody>
</table>

Cutting DNA into fragments

One class of enzymes can cut double-stranded DNA into a reproducible number of fragments. These enzymes are called restriction enzymes or cutting enzymes. These cutting enzymes occur naturally in microbes, mainly in bacteria, but also in some archaea.

The main feature of cutting enzymes is that they do not snip the two strands of a DNA molecule at random. Each cutting enzyme snips DNA at certain positions only, and is restricted to cutting at specific locations only. (This is why enzymes in this class are called restriction enzymes.)

Each cutting enzyme has its own name. These are strange-looking names such as Bgl I, Hap II, and Taq I, which are pronounced as ‘Buggle one,’ Hap two’ and ‘Tack one.’ The box below explains how the names of cutting enzymes are organised.
What is significant about restriction enzymes is that their actions on DNA are predictable as they cut DNA only at their specific restriction sites, and they act in the same way on every occasion. Before the first restriction enzyme was discovered in 1970, it was not possible to cut DNA into fragments in predictable and reproducible ways.

**ECO RI — WHAT’S IN A NAME?**

The names of cutting (restriction) enzymes look very strange indeed...

- Hap II, Eco RI, Bgl II, Not I, Pst I Hin dIII

The name of a restriction enzyme has two parts. The first part is a set of three letters, such as Eco or Bgl or Pst. The three letters are derived from the scientific name of the microbe in which the restriction enzyme occurs.

- The first letter is the first letter of the genus and the next two letters are the leading letters of the trivial (specific) name:
  - Eco is a restriction enzyme found in *Escherichia coli*.
  - Bgl is a restriction enzyme found in *Bacillus globigii*.
  - Hin is a restriction enzyme found in *Haemophilus influenzae*.

The second part of the name is a Roman numeral such as I or II or III. What does this mean? A particular species of microbe may contain several restriction enzymes. Each different restriction enzyme from the same microbe is given a Roman numeral as follows: I for the first enzyme isolated from that species, II for the second, and so on.

In some cases an additional letter is added, such as in Hin dIII, and the letter ‘d’ denotes a particular strain or type (serotype) of the bacterium.

The position where a cutting enzyme can snip double-stranded DNA is the recognition sequence of that restriction enzyme. A restriction site is a particular order of nucleotides (see figure 13.6). Some restriction enzymes cut the two strands of a DNA molecule at points directly opposite each other to produce cut ends that are ‘blunt’. Other cutting enzymes cut one strand at one point, but cut the second strand at a point that is not directly opposite. The overhanging cut ends made by these cutting enzymes are called ‘sticky’ or ‘protruding’. These sticky ends are complementary.

The expected distance between cut sites of a restriction enzyme is given by the formula $4^n$, where $n$ is the number of bps in the restriction site.

**FIGURE 13.6** Cutting by Aha I, Hae III and Hin dIII. Which restriction enzymes produce ‘sticky ends’? Are the sticky ends produced by Hin dIII complementary?

<table>
<thead>
<tr>
<th>Cutting enzyme</th>
<th>Recognition site</th>
<th>Result of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aha I</td>
<td>TTT AAA</td>
<td>[A]</td>
</tr>
<tr>
<td></td>
<td>AAA TTT</td>
<td>[A]</td>
</tr>
<tr>
<td>Hae III</td>
<td>GGCC</td>
<td>[A]</td>
</tr>
<tr>
<td></td>
<td>CCGG</td>
<td>[A]</td>
</tr>
<tr>
<td>Hin dIII</td>
<td>AAGCTT</td>
<td>[A]</td>
</tr>
<tr>
<td></td>
<td>TTCGAA</td>
<td>[A]</td>
</tr>
</tbody>
</table>

The number of nucleotide base pairs (bps) in a restriction site varies from four to about eight base pairs (bps). This length determines how frequently a restriction enzyme is likely to cut a random sequence of DNA, or in other words, the average distance between cuts. For example, restriction enzymes with a 4-bp recognition sequence, such as Hae III, are expected on average to
Restriction enzymes in common use and their recognition sites. Table 13.2 below shows some commonly used restriction enzymes.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Recognition site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alu I</td>
<td>Arthrobacter luteus</td>
<td>AG&lt;CT TC&lt;GA</td>
</tr>
<tr>
<td>Bam HI</td>
<td>Bacillus amyloliquefaciens H</td>
<td>G’GATC C CCTAG’G</td>
</tr>
<tr>
<td>Bcl I</td>
<td>Bacillus caldolyticus</td>
<td>T’GATC A ACTAG’T</td>
</tr>
<tr>
<td>Bgl II</td>
<td>Bacillus globigii</td>
<td>A’GATC T TCTAG A</td>
</tr>
<tr>
<td>Eco RI</td>
<td>Escherichia coli R factor</td>
<td>G’AATT C CTTAA G</td>
</tr>
<tr>
<td>Hae III</td>
<td>Hemophilus aegyptus</td>
<td>GG’CC CC’GG</td>
</tr>
<tr>
<td>Hind III</td>
<td>Hemophilus influenzae Rd</td>
<td>A’AGCT T TCGA’A</td>
</tr>
<tr>
<td>Kpn I</td>
<td>Klebsiella pneumoniae</td>
<td>G GTAC’C C’CATG’G</td>
</tr>
<tr>
<td>Not I</td>
<td>Norcadia otitidis-caviarum</td>
<td>GC’GGCC GC CGCCG’CG</td>
</tr>
<tr>
<td>Pst I</td>
<td>Providencia stuartii</td>
<td>C TGCA’G G’ACGT C</td>
</tr>
<tr>
<td>Sac I</td>
<td>Streptomyces achromogenes</td>
<td>GAG CTC CTC GAG</td>
</tr>
<tr>
<td>Taq I</td>
<td>Thermus aquaticus</td>
<td>T’CG A AGC’T</td>
</tr>
</tbody>
</table>

For cutting, a sample of DNA is dissolved and the particular restriction enzyme is added. Provided recognition sites are present in the DNA sample, the DNA molecules will be cut into two or more fragments. The lengths of the fragments depend on the relative positions of the recognition sites (see figure 13.8). In some cases, two different restriction enzymes may be added to a DNA sample. How, if at all, would the resulting fragments be expected to change when two restriction enzymes are used, as compared to just one?
Sorting DNA fragments

OK, so now we have a solution containing DNA that has been cut into a number of predictable fragments using a restriction enzyme. How can we sort out this mixture of DNA fragments? Sorting — no problem: use electrophoresis.

DNA fragments can be sorted using the technique of *electrophoresis*. Electrophoresis sorts DNA fragments according to their lengths, that is, their sizes.

To separate a mixture of dissolved DNA fragments, the mixture is placed in a slot at one end of a slab of jelly-like supporting material, known as a gel, that is immersed in a buffer solution. The gel, which is made of agarose, is then exposed to an electric field, with the positive (+) pole at the far end and the negative (−) pole at the starting end or origin.

Look at figure 13.9, which shows a researcher using a micropipette to load a sample of DNA into one slot at the cathode end of a gel that is covered by a buffer solution. Typically eight to ten different samples of DNA can be loaded onto one gel, with each sample being loaded into a different slot. If you look carefully, you will see blue colouring, which indicates that DNA samples have been loaded into almost all the slots. The colour is added simply to assist the researcher to load the DNA samples into the gel slots.
All DNA fragments have a net negative charge, because of the phosphate groups in their sugar-phosphate backbone. As a result, DNA fragments will move towards the positive pole when placed in an electric field. The shortest DNA fragments move most quickly and the longest fragments move most slowly. Fragments of the same size move at the same rate. The end result of electrophoresis is a series of parallel bands of DNA fragments at differing distances down the gel. (see figure 13.10). Each band can contain thousands or millions of DNA molecules of the same size.

DNA fragments are invisible so, after the gel run is complete, the separated DNA bands must be made visible either through the use of a dye or a labelled probe. One technique makes use of the dye ethidium bromide (EtBr), which binds to the major groove of DNA molecules. When illuminated by ultraviolet light, the DNA bound to EtBr fluoresces pale pink (see figure 13.11).

A standard ladder of DNA fragments of known sizes is usually run through the gel at the same time as the unknown DNA samples. The sizes of unknown DNA fragments can be approximated by comparing the positions of their bands with those of the known standards. Figure 13.11 shows a ladder of DNA markers of known sizes in lane 1, at the left-hand side of the gel.

### Interpreting gel runs

Let’s look at examples of electrophoresis in action:

**Example 1:** One of the main reasons for using electrophoresis to separate DNA fragments is to obtain estimates of the size of the DNA fragments in the mixture. Remember that electrophoresis separates DNA fragments according to their sizes. DNA fragments of unknown sizes can be separated by electrophoresis and then their sizes can be approximated by comparing them with DNA molecules of known size separated on the same gel and under the same conditions.

Examine figure 13.12, which shows a gel run of a mixture of DNA fragments of unknown sizes from the RH slot, and samples of DNA markers of known size from the LH slot. Note that the unknown sample of DNA has resolved into four bands.
The ladder of marker DNAs of known sizes shows values ranging from 23 130 base pairs to 564 base pairs. Estimate the size of the DNA fragments that have separated into the four bands. Remember that this is just an approximation.

**FIGURE 13.12** Diagram showing the separation of DNA fragments of unknown sizes on a gel (at RH side) alongside a separation of DNA markers of known sizes (at LH side). This technique is used to obtain estimates of the sizes of unknown DNA fragments in a band by comparing their positions with those of bands of DNA markers of known sizes.

**Example 2:** Samples of the DNA of the F8C gene, which controls blood clotting, were collected from several different people, two males (J and T) and one female (K).

The F8C gene is located on the X chromosome and has two alleles: the C allele that determines normal blood clotting, and the c allele that determines defective blood clotting (haemophilia).

The DNA of each sample was treated with the restriction enzyme Bcl I.

The DNA samples were then subjected to electrophoresis and the positions of the bands were identified using a fluorescent probe. The result is shown in figure 13.13b.

Let’s consider the results. Remember that the only bands visible are those labelled by the fluorescent probe. Remember that females have two X chromosomes, but males have only a single X chromosome.

You can estimate the sizes of the fragments by comparing their positions with those of the known standards.

How many fragments would result from Bcl I digestion of the C allele? (Answer: 1)

How many fragments would be produced by Bcl I treatment of the c allele? (Answer: 2)

What are their expected sizes? (Answer: 879 and 286 bp)

What has happened to the smaller 286 bp fragment? (Answer: The fragment is there, but it is not visible because the probe does not pair with it.)
Note that person K, a female, has both the C allele and c allele, as shown by the presence of two fragments.

Would all females be expected to show this pattern? (No. Most females would be expected to be genotype CC and show just one band, the 879 band.)

Person J, a male, has the C allele only. However, person T, a male, shows the presence of the relatively shorter c allele and so has the X-linked disorder, haemophilia.

![Diagram](image)

**FIGURE 13.14** In both (a) and (b), the DNA fragments have sticky ends created with the same cutting enzyme. Ligase enzyme forms strong covalent bonds so that the fragments are joined permanently. Why is the result a circular DNA molecule in (b)?

**Joining DNA fragments**

In gene manipulation, the joining of pieces of DNA is sometimes required. An enzyme known as ligase catalyses the joining of pieces of double-stranded DNA at their sugar–phosphate backbones. The bonds that form in this case are strong (covalent) bonds. The joining can produce one longer piece of DNA (see figure 13.14a) or it can produce a circular molecule of DNA if the fragments have complementary ‘sticky ends’ at both ends (see figure 13.14b).

**KEY IDEAS**

- DNA can be manipulated in various ways, including by being cut into fragments, and DNA fragments can be sorted and can be joined.
- Cutting or restriction enzymes cut DNA into fragments at specific recognition sites.
- Cuts of double-stranded DNA by some restriction enzymes produce ‘sticky’ ends, and some produce blunt ends.
- Electrophoresis sorts DNA fragments according to their lengths, measured in base pairs.
- DNA fragments can be joined through the action of the enzyme ligase.
More tools for manipulating DNA

Let’s now identify other tools and procedures that are used in DNA manipulation.

Finding DNA fragments of interest

Electrophoresis can separate a mixture of DNA fragments according to differences in their sizes, but how can one particular set of DNA fragments be picked out from a large number of DNA fragments? This is a bit like finding a needle in a haystack. However, this can be done using a probe.

You might use a person’s dog to locate that person in a crowd. In this case, the person is the target object and the dog is the ‘probe’ that locates the target. You could use a Geiger counter to find the source of a radioactive emission. In this case the probe is the Geiger counter and the target that it will locate is the source of a radioactive emission. In both cases, the probe will home in on the target. Follow the probe and you have found your target! Likewise, as people pass through security checks at airports, both their bodies and their luggage are ‘probed’ by scanners that detect targets, including prohibited metal objects, such as knives or guns. In this case, the ‘probe’ does not physically attach to the target but identifies it on a screen display (see figure 13.15).

Likewise, a molecular probe can locate particular target DNA fragments from among a mixture of many DNA fragments. This kind of probe is commonly a piece of single-stranded nucleic acid, either DNA or RNA, with a base sequence that is complementary to part of the base sequence of one of the strands.

FIGURE 13.15 The luggage scanner is like a probe that can home in on particular prohibited targets without making physical contact with the targets.

QUICK CHECK

3 Identify the following statements as true or false:
   a. Restriction enzymes cut DNA molecules at random sites along their length.
   b. The restriction enzyme Aha I produces DNA fragments with sticky ends.
   c. Electrophoresis separates DNA fragments according to their different charges.
   d. In electrophoresis, DNA fragments travel from slots at the origin of a gel to the cathode.
   e. A restriction enzyme that can act on human DNA would not be able to act on mouse DNA.
   f. In a long DNA sequence, it is reasonable to predict that there would be more cut sites for Hae III than for Hind III.
   g. In electrophoresis, shorter fragments of DNA travel farther than longer fragments.

Restriction enzymes cut DNA molecules at random sites along their length. The restriction enzyme Aha I produces DNA fragments with sticky ends. Electrophoresis separates DNA fragments according to their different charges. In electrophoresis, DNA fragments travel from slots at the origin of a gel to the cathode. A restriction enzyme that can act on human DNA would not be able to act on mouse DNA. In a long DNA sequence, it is reasonable to predict that there would be more cut sites for Hae III than for Hind III. In electrophoresis, shorter fragments of DNA travel farther than longer fragments.
of the target DNA. The key to the use of probes is their ability to hybridise with the complementary sequences on their single-stranded target DNA.

To locate the DNA fragments of interest, the target DNA must first be denatured to separate its two strands, otherwise the probe cannot home in and pair with it. Figure 13.16 shows a probe and part of the target it can locate. Because the probe is complementary to part of the target, it is able to pair with it.

**Probes**

The DNA target is dissociated into single strands:

```
...ACATTATAGGGGCTGGTTACC...
```

A probe is added and pairs with the complementary region of the target:

```
...TGAAATAATCCGCCGACCAATGG...
```

**A probe**

A probe must be labelled in some way so that it can be easily located. This label is typically a fluorescent marker or it may be a radioactive marker. The position of the probe and the target that it has latched onto are signalled by the fluorescent label on the probe.

Probes can be used to locate specific DNA segments, either within a cell or outside cells, such as on a gel.

Figure 13.17 shows the chromosomes of a human cell that have been exposed to a probe that binds specifically to a unique sequence of DNA in part of the human Y chromosome. This probe has a label that fluoresces bright yellow when exposed to ultraviolet light. Notice the two bright yellow spots that indicate that the probe has hybridised at two sites, signalling the presence of two Y chromosomes in the cell. This process is known as in situ hybridisation. The term in situ means ‘in place’ and identifies the fact that the DNA is in its normal place in the chromosomes. In fact, these cells came from a male who has 47 chromosomes, including one X and two Y chromosomes, in each of his somatic cells.

**ODD FACT**

When in situ hybridisation is carried out using a probe with a fluorescent label, the technique is referred to as **FISH** (fluorescence in situ hybridisation).
Probes can also be used to locate a particular DNA fragment from a mixture of fragments that has been sorted by electrophoresis. Figure 13.18a outlines the procedure called 'Southern blotting' in which the DNA fragments are transferred from the gel to a nylon membrane.

The steps in this procedure are as follows:

- The gel is treated with a denaturing agent to make the DNA single stranded.
- The gel is transferred to a salt solution, and a nylon membrane is placed over the gel.
- Layers of absorbent paper, weighted on top, are placed over the membrane, and the salt solution is drawn through the gel, carrying the DNA that is absorbed by the nylon membrane (see figure 13.18b for details). This procedure typically lasts for about 18 hours and, by the end of this time, all the DNA from the gel has been transferred to the nylon membrane.
- The membrane is then soaked in a solution containing a labelled probe that consists of a single-stranded nucleic acid probe that binds specifically to the intended DNA target.
- The probe, and hence the DNA of interest, is located, either by use of X-ray film in the case of a radioactively labelled probe, or by fluorescence in the case of a probe with a fluorescent label.

**FIGURE 13.18** (a) Steps in the use of a probe to locate a particular DNA target after electrophoresis. (b) Details of arrangement for the transfer of denatured (single-stranded) DNA from the gel to a nylon or nitrocellulose membrane.

### Making DNA fragments

It is easy to isolate the total DNA from human somatic cells. This, however, consists of the DNA of two sets of 23 chromosomes, each set containing about 3000 million base pairs encoding about 21 000 genes, as well as very long sequences of noncoding DNA. Some scientists may want short lengths of DNA to act as probes. Other scientists might want copies of just one specific sequence.
DNA base sequence as part of their research. How can particular segments of DNA be obtained for these various purposes?

Two methods for making specific DNA fragments are outlined below:

1. **Synthesise DNA from nucleotide building blocks.** This method makes use of an instrument called a DNA synthesiser (see figure 13.19 for one example). To use this method, the base sequence of the required DNA must be known. Instruments called DNA synthesisers can join nucleotide sub-units in a predefined order to produce DNA segments with lengths greater than 100 bases. The chemical synthesis of DNA does not require a template strand nor does it require the enzyme DNA polymerase. The products can be used as primers or as probes.

![A scientist working in a DNA laboratory. DNA synthesisers can generate primers and probes as well as lengths of DNA up to about 100 bps. DNA synthesisers work in conjunction with a computer and DNA synthesis software.](image1)

2. **Make a copy of DNA using an mRNA template.** To do this, mRNA is isolated from the specific cells in which the gene concerned is active. The enzyme **reverse transcriptase** uses the mRNA as a template to build a single-stranded DNA with a complementary base sequence. This DNA is known as **complementary DNA (cDNA)**. The reverse transcriptase procedure has been used successfully to make copies of the human growth hormone gene and also the gene for tissue plasminogen activator (t-PA), a blood clot-dissolving enzyme used in the treatment of some forms of heart attack.

   Figure 13.20a outlines a simplified version of the process to produce single-stranded cDNA using reverse transcriptase enzyme and an mRNA template. Commercial kits are available for the use of reverse transcriptase to produce cDNA (see figure 13.20b).

   To make this DNA double stranded, the enzyme DNA polymerase is then used. Note that this method will work only for coding DNA: that is, genes that produce mRNA. This method cannot be used for the bulk of the genome that consists of noncoding DNA.
mRNA isolated from cytoplasm of specific cells.

Poly-A tail added to this mRNA. (This segment provides an anchor to which a ‘primer’ can attach.)

Oligo-dT primer added and binds to poly-A mRNA tail. Reverse transcriptase enzyme added, DNA lengthens by addition of nucleotides. The order is controlled by the sequence in the mRNA.

When DNA chain is complete, mRNA is removed by alkali treatment.

Polymerase enzyme added. This catalyses the building of complementary DNA strand.

Final double-stranded DNA product (cDNA).

Amplifying DNA fragments

In forensic investigations, critical evidence can come from trace amounts of DNA obtained from a single root hair, a licked stamp, a dried blood stain, a discarded cigarette butt, a piece of chewing gum, drinking glasses, a soiled tissue or a trace of semen. For medical purposes, a few fetal cells obtained from amniotic fluid may be the source of DNA that can reveal if a fetus has an inherited disease. These DNA traces become useful when they are amplified to millions of copies.

Figure 13.21 shows an instrument that automates the PCR procedure. Minute quantities of DNA provide the starting point for PCR. From a few starting copies of DNA, millions of copies can be quickly produced.
The polymerase chain reaction (see figure 13.22) depends on the enzyme DNA polymerase to amplify or make multiple copies of a sample of DNA. The polymerase enzyme comes from a bacterial species (Thermus aquaticus) that lives in hot springs. It is known as the Taq polymerase.

The procedure is carried out in a test tube (in vitro) as follows:

1. **Denature**: The DNA sample is denatured by heating so that it dissociates to single strands (94°C for two minutes).
2. **Bind primers**: Short segments of single-stranded DNA, known as primers, are added; these primers pair with regions at either end of the DNA region of interest (55°C for two minutes).
3. **Extend primers**: The polymerase enzyme uses the primers as a starting point and extends them so that two complete double strands are formed. For this step, a supply of nucleotides must be available (72°C for one minute).

About five minutes is required for each cycle of ‘denature — bind primers — extend primers’. Over time, an exponential growth of the DNA from the two original strands occurs: 4, 8, 16, 32…, so that after 20 cycles about one million copies of the DNA exist.

The amplified DNA produced in a few hours by PCR is enough to provide material for a DNA profile in a forensic investigation, or to identify whether an embryo or fetus has inherited a genotype that causes a serious disorder, such as sickle-cell anaemia. In contrast, other prenatal procedures require several days or longer to obtain enough DNA by cell reproduction.
Plasmids: vectors of DNA

What are plasmids? In addition to their main chromosome, most bacterial cells contain one or more plasmids. Plasmids are small circular pieces of double-stranded DNA ranging in length from about 2000 to 10 000 base pairs (see figure 13.23) found mainly in bacterial cells. All plasmids are self-replicating because they contain an origin of replication (ORI) that is a specific DNA base sequence where DNA replication of a plasmid begins. As a result, plasmids can replicate independently of the main bacterial chromosome.

In addition to naturally occurring plasmids, plasmids can also be constructed so that they include certain features in addition to an origin of replication. For example, plasmids that are used to transfer DNA include several features, including:

1. genes for one or more traits, including a selectable marker gene
   The marker gene is often a gene governing antibiotic resistance, with the particular form of the gene being the allele for resistance to an antibiotic, such as ampicillin (AmpR). Bacteria containing the plasmid with the marker can be selected from among other bacteria by exposure to the antibiotic. Bacteria that have taken up the plasmid with the marker are unaffected by the antibiotic, but bacteria lacking that particular plasmid will be killed by the antibiotic.

2. a multiple cloning site (MCS), also known as a polylinker
   This is a region of the plasmid DNA that contains the recognition sites for several restriction enzymes that are ones producing ‘sticky’ ends. These are sites where it is possible to insert foreign DNA. Treatment of plasmids with the specific restriction enzyme cuts the plasmid DNA open, allowing the incorporation of foreign DNA with complementary sticky ends.
   Figure 13.24 shows two of many plasmids that can be used as vectors to transport foreign DNA into bacterial cells to transform them. Both plasmids have the required origin of replication (ORI) and also have a marker (AmpR) for the antibiotic ampicillin. In addition, the pUC19 plasmid has a second marker (TetR) for a second antibiotic, tetracycline.
Bacterial transformation

In the laboratory, scientists commonly use a plasmid as the carrier or vector to introduce new genes into bacterial cells. When foreign DNA is directly taken up from an external source by bacterial cells, those bacteria are said to be transformed. Evidence that bacterial transformation has occurred is seen in the expression of the foreign marker gene in the phenotype of the bacteria. For example, some bacteria are susceptible to a particular antibiotic, such as ampicillin. If these bacteria acquire a plasmid carrying a gene for ampicillin resistance, these bacteria are transformed and become ampicillin resistant.

Bacterial transformation may occur naturally, as when bacteria swap plasmids among themselves. Naturally occurring transformation of bacterial species by plasmid transfer has produced increasing numbers of strains of bacteria that are resistant to multiple antibiotics. Bacterial transformation may also occur artificially, as a deliberate process carried out by microbiologists in laboratories.

Bacterial transformation can involve the direct uptake by bacteria of naked fragments of DNA from the environment, such as DNA fragments from dead bacteria. Very commonly, however, transformation is achieved when vectors, such as plasmids, introduce foreign DNA into bacterial cells.

First knowledge of bacterial transformation

Transformation of bacteria by natural processes has probably been taking place for at least a thousand million years or so. However, it was not until 1928 that it was recognised — this by an English scientist, Frederick Griffith (1877–1941). Griffith experimented with pneumococcus bacteria; the smooth variety of these bacteria causes pneumonia, but the rough variety is harmless. Griffith showed that when the contents of dead smooth bacteria were added to a culture of living rough bacteria cells, some of the rough cells were transformed into pneumonia-causing smooth cells that, when injected into mice, killed them. At that time, Griffith called the agent responsible for this change the ‘transforming factor’ but its identity was not known. It was not until 1944 that Oswald Avery (1877–1955) demonstrated that the transforming factor was DNA.

Making recombinant plasmids

Recombinant plasmids are plasmids that carry foreign DNA. Making recombinant plasmids involves the following steps:

1. The DNA of the plasmid is cut at one point using a specific cutting enzyme that creates sticky ends. This changes the form of the plasmid from circular to linear.

2. The foreign DNA fragments are prepared using the same cutting enzyme so that the foreign DNA has sticky ends that match those of the cut plasmid.

   The foreign DNA fragments and the plasmids are mixed, and, in some cases, their ‘sticky ends’ pair by using weak hydrogen bonds. These are recombinant plasmids.

   Other pairings will also occur, such as cut plasmids resealing themselves so that they are not recombinant plasmids.

3. The joining enzyme, ligase, is added and this makes the joins permanent through covalent bonding.

   Figure 13.25 shows the process by which recombinant plasmids are formed.
**ODD FACT**

Smooth pneumococcus bacteria have an external capsule that prevents their destruction by phagocytic cells of the immune system. Infection by a single smooth cell can lead to the death of a mouse by pneumonia, while an infection by hundreds of rough cells that lack capsules is harmless.

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**FIGURE 13.25** Diagram showing the steps in the formation of a recombinant plasmid. The formation of complementary sticky ends in both the foreign DNA fragment and the cut plasmid means that pairing between the plasmid and the foreign DNA can occur.

In reality, commercial companies exist that will produce DNA plasmids at the highest quality to meet the design requirements of research groups, as, for example, PlasmidFactory®, a German company founded in 2000 (see figure 13.26).
Getting plasmids into bacterial cells

Once recombinant plasmids have been created, the challenge is to transfer them into bacterial cells. In general, the success rate for the transfer of recombinant plasmids into bacterial cells is low, but the success rate can be increased through various techniques:

- One technique is termed electroporation. In this method, cells are briefly placed in an electric field that shocks them and appears to create holes in their plasma membranes so that plasmid entry is facilitated.
- Another method is to heat shock the bacterial cells by suspending them in an ice-cold salt solution, and then transferring them to 42 °C for less than one minute. This treatment appears to increase the fluidity of the plasma membranes of the bacterial cells and increases the chance of uptake of plasmids.

An example of the heat-shock treatment is shown in figure 13.27. The starting culture of bacterial cells is sensitive to the antibiotic tetracycline, and is denoted Tet<sup>a</sup>.

1. The bacterial culture is placed in an ice bath and chilled.
2. Recombinant plasmids with the tetracycline resistance allele Tet<sup>b</sup> are added to the bacterial culture and chilled.
3. The bacteria and plasmid mix is placed in hot water at 42 °C for 50 seconds, producing a heat shock. This is the stage when the plasma membranes of the bacterial cells are altered, increasing the chance of uptake of plasmids by the cells.
4. The mix is returned to an ice bath for two minutes.
5. The bacteria are plated on an agar plate containing the antibiotic tetracycline and incubated at 38 °C overnight. Bacteria that have not taken up the plasmids are killed by the tetracycline. Bacterial cells that have taken up the plasmids will be selected; these bacteria have been transformed and will replicate.

**FIGURE 13.27** Stages in the uptake of plasmids by bacterial cells, resulting in their transformation.
The importance of recombinant plasmids is that the foreign DNA they incorporate can come from any source: it may be a human gene, a plant gene, a jellyfish gene, a yeast gene, and so on. The importance of transformed bacteria is that they express the foreign gene in their phenotype. We will see in chapter 14 how these transformed bacteria can be factories to produce the protein products of these foreign genes, such as human insulin.

Now that you have met plasmids, let’s look at how they can be used to make multiple copies of DNA.

Making multiple copies of DNA

It is possible to obtain multiple copies of DNA using the polymerase chain reaction (PCR) as described earlier in the chapter. However, the PCR technique is used to amplify DNA only when very tiny amounts of DNA are available, such as in forensic investigations.

In contrast, if DNA is available in relatively large amounts, recombinant plasmids can be used to make multiple copies of large fragments of DNA or even entire genes. This technique is called gene cloning and we will explore this technique in detail in chapter 14 (see page 652). Note that gene cloning using recombinant plasmids uses the fact that plasmids are self-replicating and can multiply independently of the main bacterial chromosome.

A simplified diagram showing how plasmids can make multiple copies of foreign DNA that they carry is shown in figure 13.28. This multiplication is achieved through (i) replication of the plasmids and (ii) binary fission (division) of the bacterial cells in which the plasmids are located.

In summary, the DNA to be copied (cloned) is joined to a bacterial plasmid and the plasmid is transferred into a bacterial cell. Once inside the bacterial cell, the plasmid and its foreign DNA multiply to produce up to 20 copies. Within about 20 minutes, the bacterial cell itself divides by binary fission to produce two daughter cells. Within these cells, the plasmids again multiply and then the bacterial cells divide again. Over a period of several hours, millions of bacterial cells and even larger numbers of copies of the foreign DNA will be produced.

**FIGURE 13.28** Gene cloning involves the formation of many copies of a particular fragment of DNA. In this case, the red region of the recombinant plasmid is the foreign DNA fragment. Multiplication of this foreign DNA involves both replication of the plasmid’s DNA within cells and binary fission of the bacterial cells. In this diagram, after one binary division of the bacterial cell and plasmid replication, how many copies of the original plasmid are present?
and debilitating disease, their ability to develop resistance to antibiotics and the many widespread plagues and epidemics that arise due to pathogenic (disease-causing) microorganisms.

As a microbiology PhD student, my thesis and lab research focuses on one particular pathogenic bacterium, *Clostridium difficile*. *Clostridium difficile* is the most common cause of antibiotic-associated hospital-acquired diarrhoea and can result in a spectrum of disease ranging from mild diarrhoea to severe and life-threatening diarrhoea, which may lead to more serious and fatal complications. Disease is mediated by two large toxins produced by *C. difficile*, which lead to cell death and inflammation. My research specifically focuses on how *C. difficile* interacts with the host during infection. From my research, we hope to better understand how the host responds to infection with *C. difficile* at the immunological level, as well as understanding other systemic disease complications that may arise during severe infection. To do so, we use models of infection to mimic human disease and analyse the pathologies, and immunological and host responses, induced by infection with *C. difficile*. We then compare this to infection with genetically engineered *C. difficile*, which lacks the ability to produce the disease-inducing toxins, in the hope of identifying key differences in host responses between both groups. Medical research is, however, not a one-man job and relies on our team of students and staff working and collaborating together to achieve our research outcomes. By working together on various aspects of *C. difficile* disease, we aim to pinpoint key pathways involved in pathology and disease progression during *C. difficile* infection, and hope to identify more suitable treatment options and markers for disease severity. Hopefully this will translate to better disease outcome and fewer fatalities from *C. difficile* infection.

**KEY IDEAS**

- Plasmids are small circular fragments of double-stranded DNA occurring naturally in bacteria.
- Plasmids are vectors that can carry foreign DNA from any source.
- Plasmids can be constructed to carry selectable marker genes, such as antibiotic resistance.
- Transformation of bacteria occurs when these cells take up DNA from an external source and express it.
- Recombinant plasmids can be used to make multiple copies of foreign DNA fragments or even genes.
QUICK CHECK

6 Identify the following statements as true or false:
   a Plasmids are the main chromosome of a bacterial cell.
   b Plasmids are self-replicating entities.
   c When bacterial cells and plasmids are mixed, all bacterial cells will be transformed by taking up the plasmids.
   d Bacteria that are ampicillin sensitive will be killed by exposure to this antibiotic.
   e If ampicillin-sensitive bacteria take up plasmids that contain Amp<sup>R</sup>, these bacteria will be transformed and will become ampicillin resistant.
   f The foreign DNA in a recombinant plasmid could come from mice or men.

7 Identify two procedures that can increase the chance of uptake of plasmids by bacteria.
The plasmid known as pAMP contains 4539 base pairs of DNA. These include an origin of replication (ORI), the Amp\(^R\) allele of the gene conferring ampicillin resistance. In addition, this plasmid includes a single occurrence of the Recoder, a fragment of 3755 base pairs that included the ORI and the Amp\(^R\) gene sequence.

1. The pAMP plasmid was treated with a mixture of the restriction enzymes Bam HI and HindIII. The result of this treatment was two fragments as follows:
   - a fragment of 784 base pairs
   - a fragment of 3755 base pairs that included the ORI and the Amp\(^R\) gene sequence.

2. Use the above information to draw a rough sketch of the pAMP plasmid and show the positions of the two recognition sites.

3. What is the function of the ORI?

   a. What evidence would indicate that these bacteria were recombinant pAMP plasmids?
   b. Make a rough sketch of the recombinant pAMP plasmid.
   c. With the enzyme ligase is involved in the formation of recombinant plasmids. Identify its role.
   d. The enzyme ligase is involved in the formation of recombinant plasmids. Identify its role.

4. Your task is to draw a gel with four lanes showing the relative positions of the bands after the electrophoresis is completed.

5. Fragments of foreign DNA with a size of 500 base pairs have ends resulting from Bam HI treatment. Copies of this foreign DNA were mixed with pAMP plasmids that had also been treated with Bam HI only. Some recombinant plasmids were formed.
   - a. Make a rough sketch of the recombinant pAMP plasmid.
   - b. Only some, but not all, plasmids became recombinant. Suggest a possible reason for this.
   - c. The enzyme ligase is involved in the formation of recombinant plasmids. Identify its role.
   - d. Recombinant plasmids were mixed with recipient bacterial cells that were ampicillin sensitive (Amp\(^R\)). Some bacterial cells took up recombinant plasmids, but others failed to do so.
      - a. Which bacteria were transformed?
      - b. What evidence would indicate that these bacteria were transformed?
      - c. Briefly explain how you could separate the transformed bacteria from those that were not transformed.

Indicate the origin of your gel that marks the position where the samples are loaded.

Number each lane.

At the side of your gel, include an arrow with a positive (+) and a negative (–) sign to show the direction of migration of the various DNA fragments.

First, draw the bands for the sample of known markers in lane 1 and show their sizes at the LH side of the gel.

Then draw the bands for the other samples and show their sizes at the RH side of the gel.
Chapter review

Key words

bacterial transformation  electrophoresis  ligase
Bcl I  electroporation  multiple cloning
Cas9 nuclease  FBC  site (MCS)
complementary  gene cloning  origin of replication
DNA (cDNA)  gene editing  (ori)
covalent  genome editing  plasmids
CRISPR-Cas9  guide RNA  polymers
DNA editing  in situ hybridisation  prelinker

Questions

1 Recognising similarities → Copy and complete the following:
Example:
Petrol : (is to) engine :: (as) food : (is to) animal
Saw : carpenter :: ................. : genetic engineer
Needle and thread : fashion designer :: ................. : genetic engineer

2 Recognising similarities → An analogy is defined as a comparison between two different things on the basis of some similarity. Statements such as ‘The circulatory system is like an irrigation system,’ and ‘The cell membrane is like the outer wall of a castle’ are examples of analogies.

a Identify a tool of the genetic engineer that might be seen as analogous to one of the following objects. Give a reason for each of your choices.
............... is like a photocopier because .................
............... is like adhesive tape because .................
............... is like a scalpel because .................

b Things compared in an analogy have some similarities, but they are not similar in every way. For each analogy, describe one important way in which the items compared differ.

3 Demonstrating knowledge → A solution contains many copies of a piece of DNA with three cutting sites for a particular restriction enzyme. This solution of DNA is treated with the restriction enzyme under controlled conditions.

a What is the expected result of this treatment?
b Is this change in DNA an example of a chemical or a physical change?

4 Applying knowledge and understanding → The action of the cutting enzyme Pst I is as follows:

| C T G C A G | C T G C A | G |
| G A C G T C | G | A C G T C |

8.4 kbp

A sample of this DNA was treated with the cutting enzyme Alu I. The cutting site for this enzyme is AGCT.

a After this treatment, the sample was subjected to electrophoresis. The result is shown in figure 13.30. The numbers denote the sizes of the fragments in kbp. What conclusion may be drawn?

5 Demonstrating knowledge and understanding → A young woman from a family with a history of haemophilia had a test that revealed that she was a carrier of the haemophilia allele. This woman later became pregnant and found out that her fetus was male. She wished to know if her fetus would be affected by haemophilia.

a Carefully explain if and how an answer might be given to her.
One DNA test for haemophilia involves the use of the restriction enzyme Bcl I.

b For this test, could Bcl I be replaced with another restriction enzyme, such as Sac I? Explain your decision.

6 Analysing data and drawing conclusions → A piece of double-stranded DNA is shown below.

| 8.4 kbp |

A sample of this DNA was treated with the cutting enzyme Alu I. The cutting site for this enzyme is AGCT.

a After this treatment, the sample was subjected to electrophoresis. The result is shown in figure 13.30. The numbers denote the sizes of the fragments in kbp. What conclusion may be drawn?
b A second sample of this DNA was treated with a different cutting enzyme, HindIII. The cutting site for this enzyme is AAGCTT. The result after electrophoresis revealed two DNA fragments of sizes 5.4 kbp and 3.0 kbp. What conclusion may be drawn?

c A third sample of this DNA was treated with yet another cutting enzyme, Not I. The result after electrophoresis revealed one fragment of 8.4 kbp. What conclusion may be drawn?

7 Demonstrating understanding ➔ One sample (S1) of DNA was cut into pieces using a cutting enzyme that produces blunt ends. A second sample (S2) of DNA was cut using a cutting enzyme that produces sticky ends. In both cases, the sizes of the fragments produced were similar. Scientist AA wanted to join the DNA pieces in sample S1. Scientist BB wanted to join the DNA pieces in sample S2. Both scientists carried out their experiments with the DNA in solution under identical conditions.

a Explain the terms sticky end and blunt end.

b What enzyme would the scientists use to catalyse the joining?

c The scientists observed that the pieces of DNA from one sample joined more quickly than those in the other sample. For which sample would the faster rate of joining be observed? Explain your choice.

8 Demonstrating knowledge ➔

a Describe one means by which a scientist can obtain a copy of a gene.

b Having obtained a copy, describe one means by which the scientist can obtain millions of copies of the gene.

9 Analysing data and applying principles ➔ Examine figure 13.31 and answer the following questions:

a Identify the lane that contains:

i the shortest fragments of DNA

ii DNA fragments that include one restriction site for Bgl II and were treated with that restriction enzyme

b DNA fragments with an approximate size of 1500 bps

c DNA fragments with a size in excess of 3000 bps.

b If this same DNA that was treated with Bgl II had instead been treated with HindIII, would the pattern of bands obtained in electrophoresis have been the same? Explain.

c A different sample of DNA was treated with a particular restriction enzyme that produced three fragments of sizes 400, 750 and 2500 bps. Consider that this DNA mixture was loaded into lane 5 of the gel shown in figure 13.31. Draw the expected pattern on completion of electrophoresis of this DNA in lane 5.

10 Demonstrating knowledge and understanding ➔ Complete the following sentences:

a In bacterial transformation, bacteria take up foreign ________.

b A vector commonly used in transformation experiments is a ________.

c For transformation to occur, the DNA taken up by bacteria must be ________ in their phenotypes.

d Sticky ends join temporarily through the formation of ________.

e Temporary joins can be made permanent through the use of ________.

f The replication of plasmids is ________ of the replication of the main bacterial chromosome.

11 Analysing data and applying principles ➔ Examine figure 13.32, which shows a PAES30 plasmid and the location of recognition sites for various restriction enzymes. This plasmid comprises 4715 bps. Note that KmR is the DNA of the allele for resistance to the antibiotic kanamycin, and MCS is a multiple cloning site for the restriction enzymes shown alongside it.
a What would be the effect of such a plasmid in terms of the number of fragments and sizes, if it was subjected to the following treatments:

i treatment with Nde I
ii treatment with Nde I plus Sac I
iii treatment with Nde I plus Sac I plus Cla I.

These plasmids are to be made recombinant by incorporating a fragment of foreign DNA. The decision needs to be made about which restriction enzyme to use for this purpose.

Student P says: ‘Use any one of them. It makes no difference.’ Student Q says: ‘No way. There are some we should definitely not use!’

b With which student do you agree? Explain your choice.

12 Discussion question

CRISPR-Cas9 technology allows for gene editing. An issue that is the subject of debate is:

Should gene editing be allowed on human cells, such as eggs, sperm and embryos, whose DNA can pass to future generations? Or, should gene editing be restricted to somatic cells where that DNA is not passed to the next generation?

Discuss this issue with your classmates.