elevate

YEAR 12 BIOLOGY

MODULE 6

LESSON FIVE

THEORY

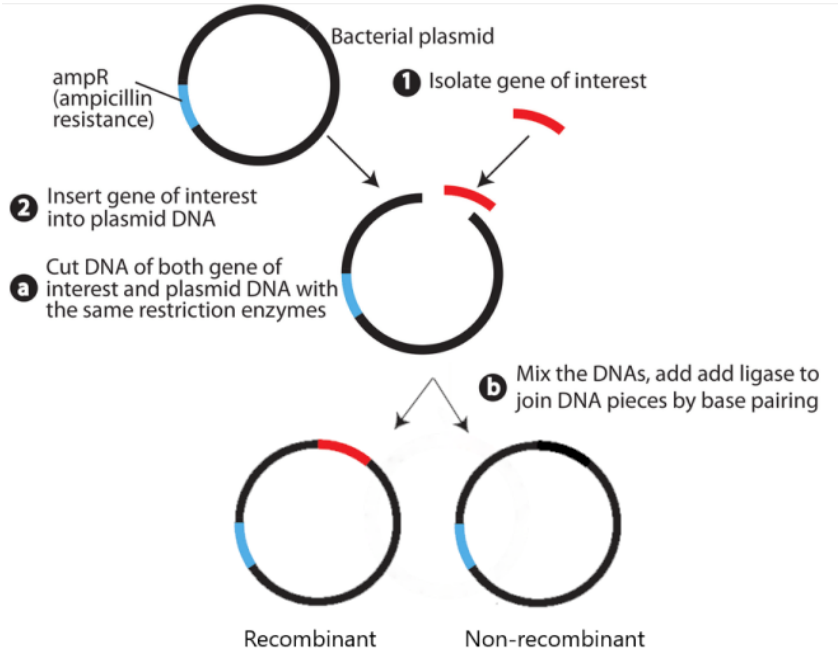
RECOMBINANT DNA TECHNOLOGY

6.3.4 describe techniques and applications used in recombinant DNA technology, for example: – the development of transgenic organisms in agricultural and medical applications

The aim of recombinant DNA technology is to introduce genes from one species into the genome of another species.

Most scientific processes to create recombinant DNA begin with the insertion of a target gene into plasmids. This technique was covered briefly last week, and so we will have a short review of it here:

1. The target gene is excised from a cell's genome using specific restriction enzymes.
2. A plasmid is removed from a bacterium and is cut open by the same restriction enzymes. Now both pieces of DNA (the target gene and plasmid) have matching sticky ends.
3. The plasmid and target gene are placed together.
4. Sometimes the sticky ends of the open plasmid and target gene bind through complementary base pairing, forming a 'recombinant plasmid'. Other times the plasmid will close back up without incorporating the target gene, forming a 'non-recombinant plasmid'.
5. DNA ligase is added to join the sugar-phosphate backbone in the regions where sticky ends bind together.



THEORY

.....

.....

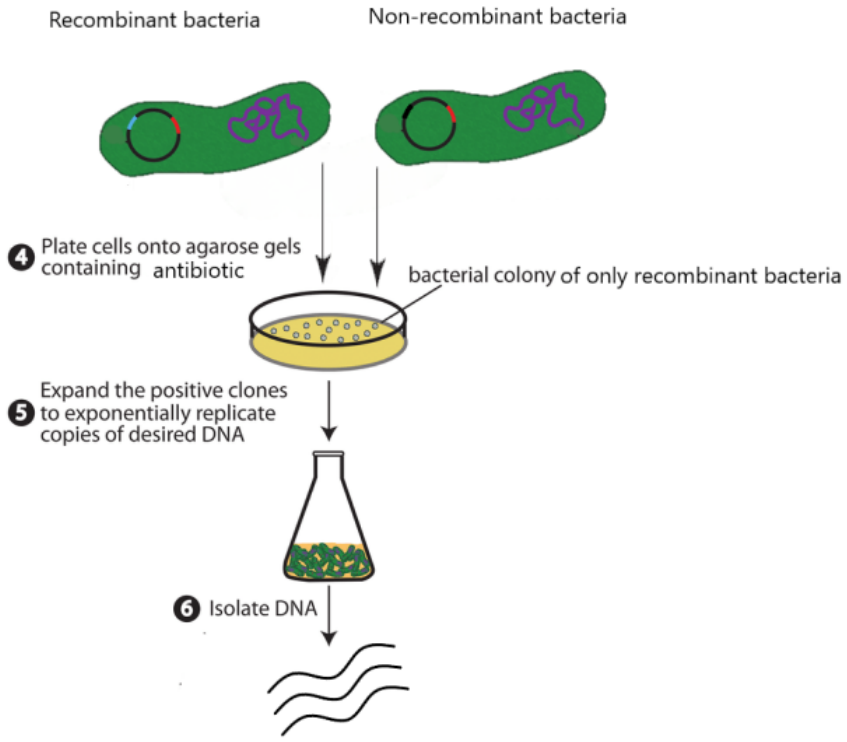
.....

.....

6. There are two methods of transformation:
    a. Heat shock - high-temperature stress opens temporary pores in bacterial cell membranes that allow plasmids to enter.
    b. Electroporation - applying an electrical current to cell membranes creates temporary pores that make it easy for foreign genetic material (i.e. the plasmid) to enter a bacterial cell

Before we move onto the cloning stage, it is important to understand that most plasmids used in recombinant DNA technology contain an antibiotic resistance gene.

7. After a bacterial culture undergoes transformation, it is placed onto an agar plate with antibiotic. Only those bacteria that took up a plasmid survive.
    a. Note that at this stage, surviving bacteria may have a recombinant OR non-recombinant plasmid.
8. After culturing the bacteria with plasmids, the presence of specific compounds can help us identify which ones have recombinant plasmids. We can sample those bacteria to make another culture that is now 100% bacteria with recombinant plasmids.
9. It is then possible to isolate the replicated target gene.



THEORY

At this stage, recombinant bacteria can simply be cultured in large vats. While the bacteria live they express the target gene in their genome and synthesise whatever polypeptide the gene codes for. The production of insulin covered last week is a great example.

However, we may also wish to transfer the plasmid, with its target gene, into eukaryotic plant or animal cells. These cells will usually be embryonic cells so that every cell in the resulting organism will contain the target gene. Some techniques for doing this include:

- Electroporation/heat shock - just like for bacteria, applying heat or current to animal or plant membranes opens up pores that allow recombinant plasmids to enter the plant cell. Once in the cell, the target gene is randomly integrated into the cell's genome.
- Micro-injection - a micropipette is used to directly inject the recombinant plasmid into the nucleus of a cell, where the target gene is incorporated into the genome.

.....

.....

.....

.....

.....

.....

CRISPR-Cas9 technology can also be used to make recombinant eukaryotic cells more precisely. One of the major limitations of all the above techniques is that the location where target genes are inserted is *random*.

CRISPR-Cas9 is a complex of enzymes that is able to target specific DNA sequences of our choosing and then cut them at a target site. We can program the enzyme to cut at specific locations in our genome. DNA repair machinery then inserts the target gene at that location.

The two main applications of recombinant DNA technology are gene cloning (covered last week) and creating transgenic organisms.

TRANSGENIC ORGANISMS

.....

.....

.....

This follows on from recombinant DNA, because the production of transgenic organisms is only possible through using recombinant DNA technology.

Both selective breeding and transgenic organisms both aim to increase the presence of favourable traits, however, the production of transgenic organisms is much faster and takes the guesswork out of it.

THEORY

GENETICALLY MODIFIED ORGANISMS (GMO) CASE STUDY

GMOs are a common topic brought up regarding genetic technologies and the ethical and social issues.

.....

.....

NB: GMO may not necessarily be a transgenic organism, because on some GMO they may remove genetics rather than add any more.

Approximately 90% of all corn, soybeans and sugar beets that we eat are GMO crops. This is because editing the genes can enable scientists to add genes that will produce crops that can taste better or resist pests or have a longer shelf life. They can also edit animal genes to have different characteristics.

Salmon Example

A company called AquaBounty engineered salmon to reach full growth in 18 months, which is half the natural time taken. This was approved to be sold in the USA and Canada.

Scientists are able to add or remove genes, or even transfer a gene from an entirely different organism into the crop to produce a new characteristic.

Currently researched GMOs:

	Researchers in the USA have found that adding a gene from yeast into tomatoes, it can increase the shelf life by 1 week. This is because the yeast gene delays the ageing and the microbial decay. This could decrease food wastage as customers only want fresh and ripe food.
	In response to the role as a vector for malaria, researchers are able to add a gene into male mosquitoes, so that when they breed, they produce offspring that don't reach adulthood, therefore limiting the population growth.
	Researchers have been able to edit cow genes to that their milk contains human proteins, and no cow proteins, which could theoretically replace human milk for babies.

All these organisms have not been released due to the potential risks.

THEORY

The most common genetic edit is the addition of pest resistance into the plant. These are called Bt crops, due to the use of a gene from the bacteria Bacillus thuringiensis. This gene causes the crop to produce the same toxin as the bacteria, to kill pests.

- This will aid the farmer as they don't need pesticides, as well as reduce the chemical exposure to the environment

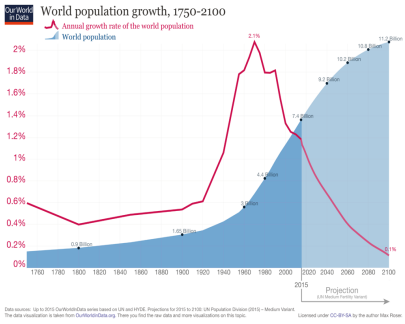
Bt Cotton Example

This cotton plant has been genetically modified to contain the toxin gene from the Bacillus thuringiensis bacteria. This works to produce the toxin in the cotton plant, to kill their common pest, the bollworm. This has been very effective so the use of pesticides has significantly reduced.

Bt Eggplant Example

In Bangladesh, eggplant farming is a major source of income. However, the bore insect can destroy up to 80% of a harvest. The introduction of Bt eggplant in Bangladesh has seen significant increases in crop and fruit size, doubling the income of these farmers.

Furthermore, the population of this world has grown significantly over the last century, and it is predicted to keep growing but plateau eventually. In this way, agricultural production needs to meet the demands of the population. The UN predicts that food supply needs to increase by 70%. Nitrogen fertilisers had a huge impact on the increase in crop size in the 20th century, but now scientists are able to genetically modify plants to increase the number of crops per seed.



THEORY

Golden Rice:

In response to the high numbers of malnutrition in developing countries, German scientists developed Golden rice, which contains a vitamin A gene, to try to combat the vitamin A deficiency in children, leading to blindness. This gene in the rice produces beta carotene which is a yellow pigment that is used by the body to synthesise vitamin A.

The intention was to make it available in places where rice is a staple food, however 20 years on from its development, most countries still haven't approved its growth, specifically the countries that need it most. Australia is one that has approved its consumption, and now the Philippines has also approved the growth and consumption.

This brings out the ethical debate of GMOs that can prevent the use of potentially beneficial usage. Greenpeace is an independent organisation that is supported by countries around the world, that brings to light environmental concerns, and they have fully rejected the use of GMOs as they fear that allowing GMOs now will only encourage the further manipulation of genetics later on. This is a valid concern that needs to be held in tension with the possible benefits.

EFFECT ON BIODIVERSITY

Adding favourable traits to an organism directly through genetic manipulation changes the process of natural selection, as humans are adding the traits, rather than arising naturally through mutations and meiosis. This gives the transgenic organisms a potentially advantageous fitness over wild species.

.....

.....

.....

.....

.....

.....

Therefore, initially, the biodiversity will increase due to the addition of a new allele into the population (transgenic organism), but if the added trait gives a selective advantage, non-transgenic species will die out, therefore significantly reducing the biodiversity in the long term.

Additionally, most crops will release their pollen allowing it to naturally fertilise other nearby plants. However, there is an added fear of transgenic organisms fertilising neighbouring farms and spreading the allele. This will further increase the allele frequency of the transgenic species therefore lowering the biodiversity, and potentially endangering wild species.

THEORY

ADVANTAGES AND DISADVANTAGES OF TRANSGENIC ORGANISMS

Advantages	Description
	Can add genes to crops to make them drought resistance or pest resistance to increase their fitness in the environment
	Can be used to produce proteins using bacteria that can be given as a treatment for diseases such as insulin for diabetes, erythropoietin for treating anaemia and growth hormone for treating growth disorders.
	The addition of pesticide genes into crops reduces the use of pesticides. Pesticides seep into waterways and can poison ecosystems, so therefore, Bt crops are helping the environment. Research is also being currently done into genetically modifying trees to absorb more carbon dioxide from the air.
Economic gain	

Disadvantages	Description
	This can occur when any transgenic species is in the wild (crops, or released animals) which can spread the transgenic gene, further increasing the allele frequency, and adding a potentially beneficial trait into some, therefore wiping out the wild species.
	The ecosystem and food chain will be affected, if a gene is inserted to target a pest. In this way, the target pest may die, or the target pest starts feeding on a non-target crop, and both will change the food chain.
	Ethical issues raised about the regulation of this technology and the ability to create mutant species, or playing God

APPLICATIONS OF TRANSGENIC ORGANISMS

Agriculture:

- Resistant crops: inserting genes that add pest (Bt crops) resistance or drought resistance enables the farmers to produce a greater healthier harvest

Industry:

- Dairy and brewing production: the dairy and brewing industry both require the use

of enzymes to speed up the fermentation process. In this way, they can use transgenic bacteria to synthesise these enzymes for mass production. These are also more pure forms of the enzymes. They can also use transgenic yeast cells which can enhance fermentation, improve sugar efficiency and altering the flavour of the final wine.

- Toxin absorption: researchers are working on producing plants and bacteria that can absorb heavy metals from the soil. For example, E. coli containing metallothionein genes from horses had high rates of mercury uptake from contaminated soil. This could aid in remediation of mine sites and other heavily polluted areas and reduce harm to local ecosystems.

Medicine:

- Treatments for disease: proteins can be synthesised via transgenic bacteria that produce human proteins that can be isolated and given as treatments
- Medical research: the creation of 'knock-out' mice has been important for research because the mouse has an existing gene removed and replaced with artificial DNA at the blastocyst stage of development. This makes the gene inactive, to observe any functional changes. This can then enable researchers to investigate treatments for genetic diseases.
- Xenotransplantation (future application): can produce transgenic pigs for the use of human organ donation, that have human complementary surface markers. This would prevent a human rejecting the organ, to meet the growing need for organs.
- Vaccine research: previously, vaccines that were used were dead or broken down parts of a pathogen (bacteria or virus), hoevere, using transgenic technology, we can isolate the particular gene that is used to make the antigens on the pathogen, and mass produce the gene (mRNA vaccines) or the antigen using the bacteria and insert them as vaccinations

Hepatitis B Vaccine

Researchers were able to isolate the gene that codes for the hep B antigen, and insert them into yeast cells, and use the host cells to mass produce the antigen. In this way, the final vaccine was only made up of the Hep B antigen, and not parts of the virus, using recombinant DNA technology.

THEORY

PRACTICE QUESTIONS

1. **A transgenic organism is one whose genome:**

    a. Has been modified using CRISPR-Cas9 technology
    b. Contains DNA which originates from another species
    c. Contains significantly mutated sequences that resemble those of another species
    d. Has been synthesised entirely in a laboratory

2. **Which of the following is NOT an effect of transgenic organisms on biodiversity?**
    a. Reduce biodiversity by outcompeting the original wild-type species
    b. Increase biodiversity by outcrossing with wild organisms, which may create new invasive or pest species
    c. Reduce biodiversity by causing pests to switch from it to a different crop
    d. Increase biodiversity because they introduce a new gene/allele into the gene pool

3. **Which is an example of a transgenic organism**
    a. A red rose crossed with a yellow rose
    b. A horse mating with a donkey
    c. A salmon containing genes from a tuna species
    d. An apple that has a gene removed

4. **Wastewater from mining sites usually contains toxic heavy metals that contaminates nearby soil and poses an environmental hazard. Researchers have been trying to create recombinant yeast, *Saccharomyces cerevisiae,* that can remove these heavy metals. Genes from two strains of bacteria were used to create recombinant yeasts. They are shown below:**

Bacterial species	Heavy metal targeted	Mechanism of heavy metal removal
Novosphingobium aromaticivorans	Arsenic	Contains a gene, called NaAE, that allows bacterium to store high concentrations of arsenic in the cytoplasm
Rhodopseudomonas palustris	Arsenic	Contains a gene, called RAE, that allows bacterium to incorporate arsenic into safe, inert metabolic products, which are then excreted out of the cell.

    a. Explain why biotechnology was needed to modify *S. cerevisiae*

(2 marks)

.....

.....

.....

.....

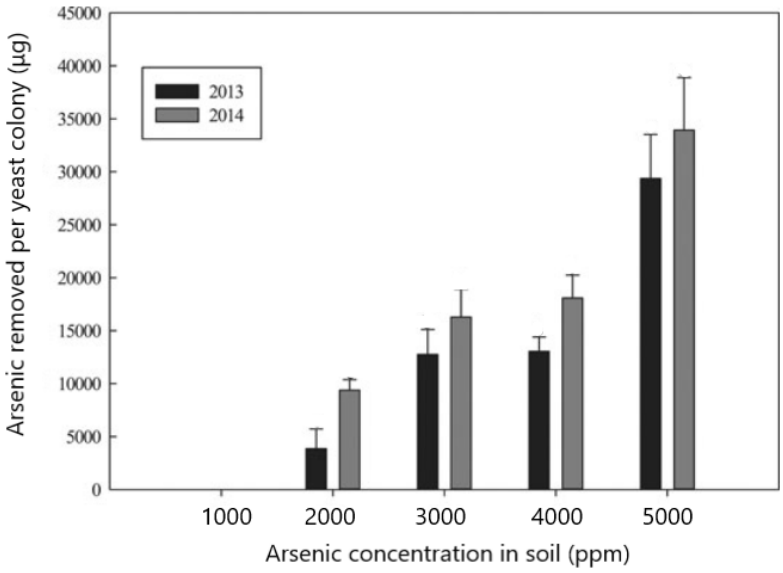
PRACTICE QUESTIONS

b. NaAE and RAE are both already located in the plasmids of their respective bacteria. Explain how these genes could be transferred from the plasmids into the genome of S. cerevisiae.

(4 marks)

c. The following graph shows the amount of arsenic removed by two strains of S. cerivisae. The 2013 strain contains NaAE while the 2014 strain contains RAE. Justify, with reference to the graph, which of the two strains would be most effective at removing arsenic from soil at mining sites.

(3 marks)



PRACTICE QUESTIONS

d. Explain ONE social and ONE economic implications of the use of the modified S. cerevisiae strains.

(4 marks)

5. An environmental organisation is protesting the allowance of a GMO wheat plant that is resistant to insects, to be grown in Australia. Explain the arguments that this environmental organisation might make against GMOs

(3 marks)

PRACTICE QUESTIONS

6. **Explain the importance of the following resources in the production of a transgenic organism:**

(3 marks)

a. Restriction enzyme

.....

.....

.....

.....

b. Bacterial plasmid

.....

.....

.....

.....

c. DNA ligase

.....

.....

7. **Using an example, explain ONE application of recombinant DNA technology in medicine.**

(3 marks)

.....

.....

.....

.....

.....

.....

.....

.....

PRACTICE QUESTIONS

HOMEWORK

1. **Which of the following is not an example of an application of transgenic organisms?**
    a. Mice with tumour-suppressor genes that have been deleted using CRISPR-Cas9 technology are used to study cancer
    b. Atlantic salmon with genes from unrelated Pacific Salmon species allow it to produce more growth hormone and reach market size faster
    c. Chickens with genes from cattle that increase the protein content of eggs
    d. Bacteria that contain plasmids with human insulin genes are used to synthesise insulin for diabetics
2. **Which of the following correctly matches the recombinant DNA technique with its meaning?**
    a. Biolistics - using a micropipette to inject DNA into the nucleus of a cell
    b. Electroporation - applying heat stress to open pores in the cell membrane through which DNA can enter
    c. Transduction - using viral vectors to deliver DNA directly into the genome
    d. Microinjection - coating DNA with metal particles and shooting it into the nucleus
3. **Why are bacteria used for creating transgenic organisms?**
    a. They divided by mitosis very rapidly
    b. They contain plasmids that can be removed and edited
    c. They have single stranded DNA that is faster to replicate
    d. They are microscopic
4. **To insert a cotton gene into a plasmid, both must:**
    a. Code for the same gene
    b. Be the same size and length
    c. Contain stop codons on either end of the gene
    d. Be cut using the same restriction enzyme
5. **How may the applications of transgenic organisms have a detrimental impact?**
    a. The creation of monocultures that are susceptible to selective pressure changes
    b. The introduction of allergens into GMO foods
    c. Damaging native flora and fauna by outcompeting them
    d. All of the above

6. **Using an example, explain ONE application of recombinant DNA technology in agriculture.**

(3 marks)

.....

.....

.....

.....

.....

.....

.....

.....

HOMEWORK

7. **Draw a flowchart showing the sequence of events that results in the creation of a recombinant plasmid.**

(4 marks)

8. **Evaluate the effect of transgenic organisms on biodiversity in the agricultural industry.**

(6 marks)

9. **Explain the short term and long term effect on biodiversity of the following transgenic organisms**
    a. Inserting a lilac gene into roses, to produce a purple rose

(4 marks)

HOMEWORK

b. Inserting a gene from bacteria into corn plants that kills pests

(4 marks)

c. Inserting a fungal gene into an endangered pine species which makes them more drought resistant, allowing them to survive in drier conditions

(4 marks)

HOMEWORK

HW ANSWERS

Lesson 4 Homework

1. D
2. B
3. D
4. B
5. A
6.

a.

1	Identifies that a plasmid was isolated from the bacteria
1	Identifies that the gene of interest from the organism was cut out using a specific restriction enzyme
1	Identifies that the plasmid is cut with the same restriction enzymes
1	Identifies that the gene is glued into the plasmid using DNA ligase which connects the sugar-phosphate backbone

b.

1	Identifies that gene A codes for antibiotic resistance, so applying antibiotic resistance will kill any bacteria that have not uptaken the plasmid
1	Identifies that gene B codes for a green protein, so any sample that produces the green protein will have a normal plasmid but not a plasmid with the gene of interest
1	Identifies that bacteria that have antibiotic resistance but don't produce the green proteins will have uptaken the correct plasmid and can be used

c.

2	Identifies an application of gene cloning and describes the use within • Pharmaceuticals • Gene therapy • Gene analysis and research
1	Identifies a relevant example • Insulin • Cystic fibrosis

HW ANSWERS

7.

a.

1	Explains that somatic cell nuclear transfer produces genetically identical offspring. This should include a very brief outline of the process of somatic cell nuclear transfer
1	Identifies that since Jango's congenital eye condition was present in his father, it's likely genetic and will be inherited by clones.
1	Identifies that a scar cannot be inherited genetically, since it involves the development of scarring tissue at a site of injury and is unrelated to germline genetic changes
1	Evaluates the statement as only somewhat accurate, since all clone troopers will have poor aim but not a scar on their nose.

b.

1	Provides a brief outline of how artificial embryo twinning works ● Egg cells that have fused with a somatic nucleus are allowed to develop into an embryo ● Before the embryo cells are specialised, the embryo is split in two ● The halves will each develop into a clone
1	Links the use of artificial embryo twinning to the logistical issue faced by clone trooper production facilities ● Since each somatic cell can now produce two embryos, more troopers can be made with the same number of somatic cells

8.

1	Define gene cloning ● Gene cloning is the process of isolating and replicating a specific gene using the polymerase chain reaction or bacteria plasmids
1	Describe an advantage ● Synthesis of human proteins via common nucleotides in DNA ● Faster and cheaper protein production using bacteria
1	Describe a disadvantage ● Challenge to identify the gene causing specific diseases, since genetics is still a relatively new field of research
1	Identifies a relevant example ● Human Insulin from bacteria

9.

1	Identifies one or more ethical advantages of whole organism cloning ● Agricultural advantages in plant cloning and propagation to maintain the life of endangered species (seed cloning) ● Cattle can be cloned to produce exact replica of the parent cell, to replicate the desirable attributes in the offspring

HW ANSWERS

1	Identifies one or more ethical disadvantage of whole organism cloning ● Cloned animals have a lower life expectancy and more health problems compared to a non-cloned animal. This can bring out animal harm ethical issues that are not justified by any significant advantages that the technology brings currently. ● Acting as God by creating identical life ● Human cloning brings out issues with identiy, specifically when attributing the actions of the clone to the original individual (criminal cases)
1	Includes an example to aid discussion ● Dolly the sheep

10.

2	Explains how gene cloning works using bacteria to produce the protein ● Gene cloning involves taking the insulin gene from the human genome using restriction enzymes. A plasmid from bacteria is then removed and cut using the same restriction enzyme to allow the insulin gene to be inserted using DNA ligase Mention restriction enzyme and DNA ligase
1	Identifies two or more advantages of using gene cloning vs pig insulin ● Less expensive ● Exact insulin protein, so more effective

11.

1	Explains that if the plasmids took up both genes, then when antibiotics were applied, it would kill off the non-recombinant plasmids, therefore only leaving the successful recombinant plasmids.
1	Identifies that this is introducing antibiotic resistance genes into bacteria, which may increase the incidence of superbugs.