19.1 Principles of Genetic Technology

Recombinant DNA

1. *define the term recombinant DNA.*

Main steps involved in creating recombinant DNA.

- Synthesise / obtain therapeutic / correct / normal / dominant allele / (c)DNA.
- From mRNA / cells of healthy person / gene library.
- Desired gene is located/ identified using probe/ electrophoresis/ sequencing.
- Gene/ DNA is amplified using PCR.
- Restriction endonuclease is used to cut therapeutic gene and plasmid/vector DNA at specific recognition sites, producing complementary sticky ends.
- DNA ligase joins therapeutic gene with vector to form recombinant DNA/ plasmid.
- Promoter sequence is added to ensure the gene is expressed (transcribed and translated) once inside target cells.

Genetic Engineering

2. *explain that genetic engineering is the deliberate manipulation of genetic material to modify specific characteristics of an organism and that this may involve transferring a gene into an organism so that the gene is expressed.*

Isolating the Desired Gene

3. *explain that genes to be transferred into an organism may be:*
   - *extracted from the DNA of a donor organism.*
   - *synthesised from the mRNA of a donor organism.*
   - *synthesised chemically from nucleotides.*

Genetic Engineering: Enzymes & Vectors

4. *explain the roles of restriction endonucleases, DNA ligase, plasmids, DNA polymerase and reverse transcriptase in the transfer of a gene into an organism.*

Enzyme	**Function**
Restriction endonuclease / restriction enzyme	Cuts vector/ plasmid DNA.
Reverse transcriptase	Makes cDNA from mRNA.
DNA ligase	Joins sugar phosphate backbone/ forms phosphodiester bonds between required gene and vector/ plasmid DNA.

Describe the roles of reverse transcriptase and DNA polymerase in making cDNA.

- reverse transcriptase uses mRNA to make single-stranded DNA.
- DNA polymerase makes DNA double-stranded.

Identify and explain the properties of plasmids that allow them to be used as vectors in gene cloning.

1. Small/ low mass: so can be inserted/enter into cells through cell membrane.
2. Replicate independently/fast OR have origin of replication OR self-replicate: So multiply / large number of plasmids / high copy number - make many copies of the gene.
3. Has restriction site(s) / can be cut by restriction enzymes: So new gene can be added.
4. Have multiple cloning site / polylinker: So can be cut by different restriction enzymes.
5. Have marker genes (antibiotic resistance/ fluorescence/ reporter genes): So recombinants / transformed bacteria can be recognised.
6. Have promoter: So gene can be expressed/ transcribed.
7. Circular: So more stable / not damaged by host cell enzymes.

Genetic Engineering: Promoters & Marker Genes

5. *explain why a promoter may have to be transferred into an organism as well as the desired gene.*
6. *explain how gene expression may be confirmed by the use of marker genes coding for fluorescent products.*

Explain why differences in the control of gene expression in prokaryotes and eukaryotes mean that expression vector plasmids must contain a prokaryotic promoter.

- Eukaryote and prokaryote promoter sequences are different.
- Eukaryote and prokaryote RNA polymerase enzymes are different.
- Prokaryotic RNA polymerase does not recognise / bind to eukaryotic promoter OR prokaryotic RNA polymerase only binds to prokaryotic promoter.
- So no transcription occurs, no mRNA is made, so no gene expression.
- Eukaryotic promoter requires binding of (many) transcription factors that are not present in prokaryotic cell.

To produce transgenic pigs, foreign DNA was injected directly into the nuclei of zygotes. The foreign DNA was made up of 2 components:

- *the gene coding for human growth hormone*
- *a section of mouse DNA that allows transcription to begin in presence of metal ions*

Suggest and explain why the mouse DNA was included in the foreign DNA.

- Mouse DNA is the promoter.
- Where RNA polymerase / transcription factor(s) bind.
- Controls / allows / ensures / is needed for gene expression / gene activation / mRNA production / hGH production.
- Gene can be switched on / transcribed by adding metal ions.
- Controls when / where / how much the gene is expressed / transcribed.

When creating a virus vector:
Viruses containing different base substitutions were tested. This was done by using the different viruses to deliver a new gene, the gene for green fluorescent protein (GFP), into photoreceptor cells of mice, using the retinal injection method. The best virus, known as 7m8, caused the photoreceptor cells in the retina of the mouse to fluoresce brightly, even when the recombinant virus was injected into the fluid inside the eye instead of into the retina itself.

Explain why the photoreceptor cells of the mouse fluoresced.

- 7m8 virus/ vector crossed fluid / reached photoreceptors / reached retina.
- Virus delivered new GFP gene/ DNA to photoreceptors / retina cells.
- GFP gene/ DNA was expressed in photoreceptors / retina cells.
- GFP/ protein product is fluorescent.

A new technique that aims to cause a **deletion** in a gene uses an enzyme called Cas9 nuclease. It is injected into zygotes along with an RNA sequence (the guide RNA) that is complementary to a target gene. The Cas9 nuclease causes a deletion in the target gene in the zygotes, preventing the expression of that gene.

The toxicity and efficiency of the new technique was tested on four groups of pig zygotes. These pig zygotes were produced by IVF using:

- ova from a female non-transgenic pig.
- sperm from a male transgenic pig whose somatic (body) cells contained one copy of the *GFP* gene per cell.

The pig zygotes in three groups were injected with different concentrations of Cas9 nuclease and guide RNA **targeted at the *GFP* gene**.

The fourth group of pig zygotes (control group) was **not** injected with Cas9 nuclease and guide RNA.

Explain why the GFP gene was chosen for testing the new technique.

- It is a marker.
- No fluorescence means GFP gene was deleted.

Some of the zygotes in each group survived and after six days each had developed into a group of cells called a blastocyst.

The blastocysts were counted using a light microscope. A filter was then added to the microscope, so that only blastocysts expressing the green fluorescent protein showed up. These were counted and the results are summarised in Table 5.1.

Table 5.1

concentration of Cas9 nuclease and guide RNA / ng mm^{-3}	number of blastocysts seen under white light	number of blastocysts seen under filter
0 (control)	68	46
10	40	0
20	24	0
50	15	0

Calculate the percentage of zygotes in the control group that were transgenic.

- (46/68) x 100 = 67.6%

Explain whether this percentage you calculated is higher or lower than expected.

- Higher, as expect 50% of offspring to get GFP gene from heterozygous male.

Name a statistical test that would allow you to test the significance of the difference between the percentage you calculated in and the expected percentage.

- χ^2 / chi-squared

State the best concentration of Cas9 nuclease and guide RNA to use to cause a deletion in the GFP gene and give reasons for your choice.

- 10 ng mm^{-3}
- more blastocysts
- less toxic
- no blastocysts seen under filter / as successful as higher concentrations / all blastocysts have deleted GFP

Genetically modified cotton contains a 'genetic package' that contains: the gene coding for Cry1Ac (Bt protein toxic to insects), a promoter, and a herbicide resistance gene that is used as a marker. Explain why a promoter is included.

- so new / foreign / inserted genes are expressed / switched on / transcribed (and translated).
- RNA polymerase binds at promoter.
- promoter directs RNA polymerase to to correct / template strand.
- to control quantity of Cry1Ac / protein made.
- to control where / which parts of plant make Cry1Ac / protein.

Gene Editing

7. *explain that gene editing is a form of genetic engineering involving the insertion, deletion or replacement of DNA at specific sites in the genome.*

	Genetic engineering using a plasmid (original technique)	**Gene editing (newer technique)**
It can produce a transgenic organism.	✓	✓
It can modify the characteristics of an organism.	✓	✓
It can delete unwanted DNA.		✓
It uses an enzyme that cuts DNA.	✓	✓
It can use RNA to precisely locate the target gene.		✓

Describe advantages of gene-editing technique compared to the traditional genetic modification technique to make transgenic pigs.

- Success rate in altering gene is greater (100% instead of 1%).
- Only specific gene is altered / targets gene more precisely.
- Unwanted gene is removed / deleted / disabled / knocked out.

A scientist stated that this new gene-editing technique is a form of selective breeding, so is not genetic engineering. Discuss whether this statement is true and whether public groups who oppose transgenic animals will be more or less likely to accept the new technique.

<u>Why not selective breeding:</u>

- Not selective breeding as DNA / genes are manipulated/ altered/ changed/ removed.
- Not selective breeding as IVF is used.
- Not selective breeding as no crossing and selection (of offspring) / repetition.
- Not genetic engineering as no new gene is put into organism / zygote.

<u>Reasons why more acceptable:</u>

- no foreign gene inserted / no cross-species gene transfer.
- only one / single gene altered / silenced.
- application reduces suffering of / viral disease in pigs.

Polymerase Chain Reaction

8. *describe and explain the steps involved in the polymerase chain reaction (PCR) to clone and amplify DNA, including the role of Taq polymerase.*

Suggest how errors occurring during PCR can cause base substitution mutations in the DNA sequence.

- Wrong base added / wrong bases pair up / base pair mismatch.
- With/ to template DNA / strand(s).
- Taq polymerase inaccurate / no proofreading.
- In the extension / elongation stage, mistake/ mutation replicated/ copied (many times).
- High temperature speeds up replication and increases chance of more mistakes.

Gel Electrophoresis

9. *describe and explain how gel electrophoresis is used to separate DNA fragments of different lengths.*

A new technique that aims to cause a **deletion** in a gene uses an enzyme called Cas9 nuclease. It is injected into zygotes along with an RNA sequence (the guide RNA) that is complementary to a target gene. The Cas9 nuclease causes a deletion in the target gene in the zygotes, preventing the expression of that gene.

The toxicity and efficiency of the new technique was tested on four groups of pig zygotes. These pig zygotes were produced by IVF using:

- ova from a female non-transgenic pig.
- sperm from a male transgenic pig whose somatic (body) cells contained one copy of the *GFP* gene per cell.

The pig zygotes in three groups were injected with different concentrations of Cas9 nuclease and guide RNA **targeted at the *GFP* gene**.

The fourth group of pig zygotes (control group) was **not** injected with Cas9 nuclease and guide RNA.

Fig. 5.1 shows the results from a second trial of the new technique, analysed by electrophoresis.

- Lanes **1–4** show DNA from four pigs born after Cas9 nuclease was used to cause a deletion in a target gene coding for a cell surface protein.
- Lane **5** shows DNA from their surrogate mother.
- Lane **6** shows DNA from another normal pig for comparison.

The size of the DNA fragments is given in kilobase pairs (kbp) as shown in Fig. 5.1.
1 kbp is 1000 base pairs of DNA.

The target gene measures 6 kbp and codes for a cell surface protein that is essential for the disease virus PRRSV to infect cells in the pig's body.

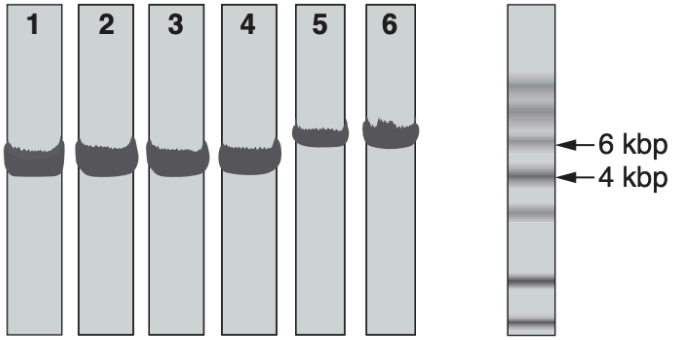

Fig. 5.1

Explain what Fig. 5.1 indicates about the success of the new technique in causing a deletion in a gene in pigs so that they show resistance to PRRSV.

- lanes 1–4 show 4 kbp fragment.
- so technique is 100% successful.
- 6 kbp gene has 2 kbp deleted / lost.
- pigs 1–4) have no normal cell surface protein.
- PRRSV / virus cannot infect the cells / pigs 1–4.

Microarrays

10. outline how microarrays are used in the analysis of genomes and in detecting mRNA in studies of gene expression.

Groups of normal mice and AD mice either received training to allow them to learn how to swim a water maze, or they received no training. The mice in the four groups then had mRNA extracted from the memory-forming areas of their brains.

Reverse transcription of the mRNA of individuals in each group was carried out and the resulting cDNA was labelled with fluorescent nucleotides. This was then used for DNA microarray analysis using slides containing DNA sequences from **33 696** mouse genes.

Explain the principles of this type of DNA microarray analysis.

- microarray identifies active / switched on / expressed / transcribed genes.

- transcription of a gene produces mRNA, which is reverse-transcribed to form cDNA, cDNA is single-stranded and fluorescently labelled.
- ssDNA act as probes / reporters.
- ssDNA bound at known positions to a solid surface / slide / chip.
- cDNA binds to / hybridises with complementary (probe) ssDNA.
- this shows up / identified as fluorescent spots / named colour.
- positions / intensity recorded by laser / scanner.
- positions identified as named genes.
- intensity of fluorescence proportional to gene expression.

Bioinformatics

11. *outline the benefits of using databases that provide information about nucleotide sequences of genes and genomes, and amino acid sequences of proteins and protein structures.*

Compared to human calcitonin, salmon calcitonin is more biologically active. Explain how bioinformatics helped identify salmon calcitonin as more biologically active and a suitable form of calcitonin to treat human osteoporosis.

- Bioinformatics is a store / database of base sequence / DNA sequence / protein / amino acid sequence data.
- These sequence data are pooled from all over the world
- It is to compare base / DNA / protein / amino acid sequence data.
- To search for base / DNA / protein / amino acid sequences similar to human calcitonin.
- It can be used for modelling / predicting tertiary / 3D / protein structure.

Explain how bioinformatics can help to identify whether the genes whose expression is changed by moving from one pattern to the other are important to health.

- Compare with known genes / sequences / genomes in the database.
- Search / analysis programme / software / algorithm.
- Identify role of proteins / (named) health effects.

19.2 Genetic Technology Applied to Medicine

Recombinant Human Proteins

1. *explain the advantages of using recombinant human proteins to treat disease, using the examples insulin, factor VIII and adenosine deaminase.*

Explain the advantages of producing human therapeutic proteins, such as insulin, by recombinant DNA technology.

- Identical to human insulin / protein.
- Lower risk of allergic reaction / immune response / side effects.
- No chance of developing tolerance to animal insulin.
- Low risk of transmitting diseases / pathogens / infection.
- Large/ unlimited supply unlimited OR large-scale mass production.
- Lower cost of production / purification / processing
- No ethical objections / no religious objections.
- Potential to engineer / improve recombinant proteins: Human protein may have higher activity / work better / (more) rapid response.

People with Alzheimer's disease (AD) lose their ability to form new memories. One form of Alzheimer's disease, called familial Alzheimer's disease, is caused by an autosomal dominant allele of the *APP* gene.

To study Alzheimer's disease, identical genetically modified mice containing the dominant human *APP* allele have been produced. These mice are known as AD mice and are used as mouse models of Alzheimer's disease.

When these AD mice are trained to swim through a water maze, they perform poorly and cannot learn as well as normal mice.

Suggest what steps will be needed to make identical genetically modified AD mice.

- obtain dominant APP allele.
- synthesise gene.
- make cDNA from mRNA.
- use probe.
- select and amplify with PCR.
- gel electrophoresis.
- restriction enzyme.
- use vector / plasmid / virus ; gene gun / direct microinjection.
- on zygote / secondary oocyte / egg cell / early embryo.
- cloning / embryo splitting.
- add promoter.
- marker gene / tag gene.

Suggest why it is useful to have an animal model of a human disease.

- to test treatments without harming humans.
- to investigate cause / progress of disease.

Genetic Screening

2. *outline the advantages of genetic screening, using the examples of breast cancer (BRCA1 and BRCA2), Huntington's disease and cystic fibrosis.*

Suggest advantages and disadvantages of screening for Huntington's disease before any symptoms occur.

Advantage

- can choose whether to have children.
- can prepare for the future.
- if negative removes anxiety.

Disadvantage

- if positive no treatment possible.
- if positive social / financial discrimination; e.g. life insurance refusal.
- if positive may still not develop disease (below 39 repeats).
- if positive may lead to anxiety.

Gene Therapy

3. *outline how genetic diseases can be treated with gene therapy, using the examples severe combined immunodeficiency (SCID) and inherited eye diseases.*

Explain what is meant by gene therapy.

- It is used to treat disease caused by faulty / recessive allele.
- gene / allele / DNA is delivered into target cells of individuals.
- named example: SCID / cystic fibrosis.

Leber's congenital amaurosis (LCA) = autosomal recessive eye disease which results in eye disorders, including severe loss of vision, at birth. LCA has been successfully treated by gene therapy, using a virus instead of a plasmid as the vector.

Explain why the fact that LCA is an autosomal recessive genetic disease makes it suitable for treatment with gene therapy.

- Can add/insert correct/ normal/ therapeutic/ dominant/ functional DNA/allele
- Only need one allele / copy per cell.
- To cure disease / correct phenotype / restore function / restore vision.
- To make / synthesise correct / functional protein.
- No need to edit / knock out / remove / delete faulty allele (as would be the case if faulty allele was dominant).

To treat LCA, adeno-associated virus (AAV) vectors containing therapeutic allele were injected directly into the retina, the layer at the back of the eye containing the photoreceptor cells. People who had been blind from a young age were able to see again. There is a risk associated with the injection method used to deliver the vectors, as it might cause the retina to detach, damaging vision.

This method of delivery was first used for LCA before being trialled on other retinal diseases that gradually reduce the vision of people as they get older – Why?

- LCA patients already blind / have lost vision / cannot see.

- Less likely to harm / add to problem / worsen vision for LCA patients.
- Other age-related diseases may involve many genes / dominant alleles.

A new emergency gene therapy treatment for people who are at risk of brain damage from a stroke was tested in mice.

- The human granulocyte colony-stimulating factor, hG-CSF, is a protein that stimulates the production of stem cells in bone marrow.
- mRNA coding for hG-CSF was obtained and used to make cDNA.
- This cDNA was inserted into an adeno-associated virus (AAV) vector and given in eye drops to mice just after they experienced a stroke.

The AAV vector used was unable to replicate itself within the target cells. Suggest why a vector that could not replicate was chosen.

- to prevent virus spreading throughout the body.
- to limit side effects / immune response / cancer / illness / infection / cell destruction.

A study was carried out to investigate the effect of the gene therapy described in **(a)**.
Four groups of mice were used.

- Group **A** mice had a stroke. They received eye drops containing AAV vector carrying cDNA for hG-CSF **once** only.
- Group **B** mice had a stroke. They received eye drops containing AAV vector carrying cDNA for hG-CSF **four** times.
- Group **C** mice had a stroke. They received eye drops containing AAV vector carrying the *GFP* gene coding for green fluorescent protein, instead of the cDNA for hG-CSF, **once** only.
- Group **D** mice did not have a stroke. They were not given any eye drops.

Explain why the mice in group C were used in the study.

- Control.
- to see if gene therapy has worked OR to compare the effect of no gene therapy with gene therapy OR to compare the effect of no hG-CSF gene with presence of hG-CSF gene.
- to see where the gene carried by the vector goes in body / brain OR to see if the gene carried by the vector enters cells OR to see where cells have been transformed.
- to see the effects of the vector / virus alone.

Explain why the mice in group D were used in the study.

- control to compare results of mice without stroke to mice with stroke.
- to establish baseline figures in mice without a stroke / act as reference point.
- to make the study valid.

Table 5.1 summarises some results from the study and shows:

- the percentage of mice surviving
- the percentage of brain volume occupied by fluid-filled space
- the score on a behavioural test in which normal mice score 0.5 and brain-damaged mice score nearer to 1.0.

Table 5.1

mouse treatment group	percentage of mice surviving	percentage of brain occupied by fluid-filled space	behavioural test score / arbitrary units
A	63	3.6	0.67
B	100	3.0	0.67
C	25	5.2	0.90
D	100	3.0	0.50

Use the results in the table to evaluate the benefits of gene therapy treatment, with AAV vector carrying the gene for hG-CSF, for people who have a stroke.

- Survival: increases survival.
- Brain damage: reduces percentage of brain occupied by fluid filled space.
- Behaviour test: improves / lowers behavioural test score; score closer to normal.
- General:
    - comparative data quote to support mp1/mp2/mp3.
    - eye drops four times is better than eye drops once.
    - if effective with mice likely to be effective with humans.
    - unknown differences between mice and humans / study is only on mice.
    - treatment is non-invasive / quick.

People with Alzheimer's disease (AD) lose their ability to form new memories. One form of Alzheimer's disease, called familial Alzheimer's disease, is caused by an autosomal dominant allele of the *APP* gene.

To study Alzheimer's disease, identical genetically modified mice containing the dominant human *APP* allele have been produced. These mice are known as AD mice and are used as mouse models of Alzheimer's disease.

When these AD mice are trained to swim through a water maze, they perform poorly and cannot learn as well as normal mice.

Researchers wanted to know if changes in gene expression were important in the inability of the AD mice to learn.

Groups of normal mice and AD mice either received training to allow them to learn how to swim a water maze, or they received no training. The mice in the four groups then had mRNA extracted from the memory-forming areas of their brains.

Reverse transcription of the mRNA of individuals in each group was carried out and the resulting cDNA was labelled with fluorescent nucleotides. This was then used for DNA microarray analysis using slides containing DNA sequences from **33 696** mouse genes.

The genes which are expressed in the brains of normal mice undergoing training and learning code for proteins important in synapse and memory formation. A large number of these genes are under the control of one transcription factor, a protein called Crtc1.

To try to improve learning in AD mice, researchers caused over-expression of the *Crtc1* gene in the brains of AD mice, by delivering the gene to mouse brain cells using a virus vector.

State the name given to this type of treatment.

- Gene therapy

Suggest the effects of over-expression in the brain of the *Crtc1* gene on AD mice.

- switches on genes needed for forming memories / synapses.
- allows better learning of water maze.
- Reduces memory loss / symptoms of Alzheimer's.

Huntington's disease is caused by a dominant allele of the gene that codes for the production of the huntingtin protein. This protein affects the development of many different tissues, including brain tissue.

- The Huntington allele contains several repeats of the base sequence CAG, which codes for glutamine.
- This results in a polyglutamine section in the synthesised protein.
- A gene with more than 39 CAG repeats produces a protein that does not fold properly and does not function.

- Symptoms of Huntington's disease usually first appear between the ages of 30 and 45 years.
- There is no treatment for the disease, which is progressive and always fatal.
- Some people with between 27 and 35 CAG repeats do not develop the disease, but may still pass on the Huntington allele to their children, who may develop the disease as the number of repeats tends to increase when gametes are produced.

With reference to the information given, explain why Huntington's disease cannot be treated with gene therapy.

- allele is dominant.
- so will still be expressed even when normal / recessive allele is present.
- gene therapy is only used to treat recessive allele disorders.
- it cannot remove dominant allele / replace an allele.
- dominant allele affects tissues in many parts of the body.

Gene Technology in Medicine

4. *discuss the social and ethical considerations of using genetic screening and gene therapy in medicine*

A couple, in which one partner has the Huntington allele, may choose to use IVF (*in vitro* fertilisation) to have a child.

Any embryos obtained from the IVF procedure can be screened in the following way:

- carry out an embryo biopsy
- use PCR
- test for the presence of the Huntington allele
- only implant embryos that do not contain the Huntington allele.

Explain why PCR is used in this procedure.

- to amplify the DNA / gene from the embryo cell.

Outline social or ethical implications of screening embryos in this way.

- embryos might be destroyed.
- wrong for parents to choose / designer embryos.
- contrary to beliefs / values.
- less chance of Huntington allele being passed on / decrease in frequency of Huntington allele.
- people w faulty allele who otherwise would not have children can now do so.

19.3 Genetically Modified Organisms in Agriculture

Genetically Modified Organisms in Agriculture

1. *explain that genetic engineering may help to solve the global demand for food by improving the quality and productivity of farmed animals and crop plants, using the examples of GM salmon, herbicide resistance in soybean and insect resistance in cotton.*

MON810 is a GMO variety of maize that has insect resistance. Outline how genetic engineering gave MON810 the trait of insect resistance.

- Gene(s) for insect resistance from another species identified (Bt gene from soil bacterium *Bacillus thuringiensis*).
- Restriction endonuclease cuts DNA to isolate gene, and cuts plasmid/vector to create complementary sticky ends.
- Gene is inserted into vector – Ti plasmid from *Agrobacterium tumefaciens* / gene gun.
- DNA ligase joins gene with plasmid DNA.
- This forms recombinant DNA / plasmid.
- Recombinant plasmid is transferred / introduced into maize cells/ genome.
- Promoter was added to ensure gene is expressed in MON810 maize cells, and marker genes were inserted to identify transformed cells.
- Grow cells on growth medium/ tissue culture / produce callus.
- New gene was expressed / transcribed and translated to make protein / toxin resistant to insects.

(same points for herbicide resistance, except the gene is not Bt gene)

Describe the difference between Bt maize and non-GM maize that explains why Bt maize is resistant to insects.

- Bt maize contains gene from Bacillus thuringiensis / bacterium.
- Bt maize produces Bt toxin / compound harmful to insects.

There are many different strains of the soil bacterium *Bacillus thuringiensis*. Each produces slightly different types of Cry-proteins, which are toxic to insects. Some types of cotton, known as Bt cotton, have been genetically modified to produce one of these proteins, Cry1Ac. This protein acts specifically to kill the larvae of butterflies and moths, including the cotton bollworm, *Helicoverpa zea*, a serious pest of cotton crops.

The genetically modified cotton contains a 'genetic package' that includes:

- the gene coding for Cry1Ac, the Bt protein
- a promoter
- a herbicide resistance gene that is used as a marker.

Suggest the advantages of using, in Bt cotton, the gene coding for Cry1Ac, rather than one of the genes coding for other types of the Cry-protein.

- only kills / targets / acts on specific / some insects / pests.
- does not kill beneficial / useful insects.
- such as pollinators / bees / predators of pests.
- to conserve / protect biodiversity / food web.
- other Cry proteins might not kill right pests / bollworm.

Suggest how herbicide resistance gene can be used as a genetic marker, when genetically modifying crops for insect-resistance.

- insert herbicide resistance gene next to Bt / Cry1Ac gene.
- spray / add herbicide on transformed plants / protoplasts / cells.
- survivors have Bt / Cry1Ac gene.
- to identify successful / GM / insect-resistant plants.

Using GMOs in Agriculture

2. *discuss the ethical and social implications of using genetically modified organisms (GMOs) in food production.*

Social benefits of gene editing of food crops

- More food / increased yield / help solve global demand for food.
- Improves quality of crop / produce / fruit.
- More income for growers / farmers.
- Spend less on / use less / no need for chemicals / pesticides.
- Cheaper / lower cost food / produce / fruit to consumers.

country	percentage change in yield of maize	
	decrease GM maize cultivation to 0% of total	increase GM maize cultivation to 88% of total
Argentina	–8.86	+2.90
Honduras	–1.26	+16.75
Spain	–3.82	+5.39
USA	–7.63	0.00

Explain what the data in the table suggests about the social and ethical implications of growing GM maize.

Description:

- Ban / 0% GM maize decreases yield / harvest.
- Growing more / 88% GM maize increases yield / harvest.

Social Implications

- GM crops increase food supply.
- GM crops decrease food cost.
- GM crops increase a country's wealth.
- With GM crops, less money is spent on insecticides.
- GM crops increase profits / improve the economy by increasing purchase price.
- GM crop improve food security, which may help prevent social unrest/ wars/ conflict that arise from food shortages.

Ethical Implications

- GM crops relieve hunger / starvation.
- GM crops may have unknown health consequences (positive or negative).
- High cost of GM seed (may disadvantage poor farmers).

Environmental implications (also comes under ethical)

- GM crops reduce land area needed for crops, conserving habitats / protecting biodiversity / allowing biofuel cultivation.
- With GM crops, less insecticides could be used (reducing environmental harm).
- GM crops may cause a decrease in genetic variation / biodiversity.
- Growing GM crops helps reduce greenhouse effect / global warming / climate change / risk of flooding.

Worldwide ban on growing GM crop varieties = more land needed to grow traditional crops. This would involve converting forest and grassland to crop-growing land.

change in use of land	change in carbon dioxide emissions /million kg CO_2
forest to crop-growing land	+ 608 726
grassland to crop-growing land	+ 276 042

Discuss what the data in the table indicates about the environmental implications of growing GM crops.

- Growing GM crops helps protect ecosystems / habitat / biodiversity.
- It helps reduce greenhouse effect / global warming / climate change / risk of flooding.

Suggest reasons why the area of land used to grow GM crops in the USA is greater than the area of land used to grow GM crops in Brazil.

USA:

- Can afford GM crops.
- Technology is more developed.
- More land available to grow crops.
- Fewer laws restricting GM crops / more widespread public approval.
- Climate conditions more suitable for GM crops.

State benefits to farmers of insect resistance in crops.

- Increased yield
- Increased quality
- Less / no pesticide / insecticide needs to be used.
- So less / no money spent on pesticides / insecticides; cheaper production.

State reasons why people may have objections to the growth of insect-resistant GM crops.

- Resistance to insects may be transferred to wild plants.
- May kill useful insects / pollinators.
- Insects may become resistant to toxin.
- May cause contamination of food marketed as organic.
- Causes decrease in biodiversity.
- Potential health risks of humans of eating GM crops.

Suggest why a smaller percentage of population object to the use of GM crops in recent years

- Education / awareness.
- Reasons for objections have not been proven.
- Consumption of GM foods shows no ill effects.
- Entire generation grown up in GM era.

Human growth hormone synthesised by transgenic pigs had the effect of making pigs grow faster, larger and heavier than non-genetically modified pigs.
Suggest reasons for this.
In transgenic pigs:

- GH concentration higher / GH always present not just at certain times.
- hGH works for longer / broken down less quickly than pig GH.
- Increase in cell signalling
- Increased / activates gene expression / transcription.
- Increased / stimulates cell division / mitosis.
- Make more muscle / bone / fat.

Only 1% of attempts successfully produced transgenic pigs. These pigs showed higher body mass and a greater muscle to fat ratio than normal pigs. Monitoring of the pigs' behaviour revealed that they rested more than normal pigs, suffered from stomach ulcers and were unwilling to mate.
Discuss whether these transgenic pigs have long term economic value.
No:

- Problem with mating, so few offspring / don't pass on gene(s) / unsustainable / assisted reproduction is expensive.
- Stomach ulcers, so high cost of healthcare / less growth / die young.
- People may avoid / refuse to buy / pay less for GM food.
- Production cost is expensive / outweighs benefits (as 1% success).

Yes:

- Greater yield / more meat (as higher body mass / muscle).
- Higher price / worth more money (as more muscle to fat ratio).
- Sell / slaughter / process earlier / at younger age.

Comment on the ethics of producing transgenic pigs showing the features described.
Unethical:

- Pigs suffer / have (stomach) ulcers / experience pain.
- Pigs cannot behave normally / move much / exercise / keep fit / socialise / mate.

Ethical:

- More /better quality meat / food for humans.
- Pigs suffer less than (normal) pigs given hGH by injection.

(b) Table 4.1 shows information about the cultivation of Bt cotton and non-GM cotton by farmers in India in 2002–2003.

Table 4.1

	Bt cotton	non-GM cotton
mean yield of cotton/kg ha^{-1}	264	196
seed cost/rupees ha^{-1}	629	196
insecticide cost/rupees ha^{-1}	503	851
net income/rupees ha^{-1}	2118	1253

With reference to Table 4.1, compare the costs involved in growing Bt cotton with the costs involved in growing non-GM cotton.

- Bt seed costs more but insecticide costs less.
- total cost is more for Bt than for non-GM.
- manipulated figures comparing both Bt and non-GM.

Table 4.1 shows that farmers who grow Bt cotton have higher net income than those who grow non-GM cotton. Use information in the table to suggest reasons why some farmers in India choose to grow non-GM cotton, rather than Bt cotton.

- non-GM seeds are cheaper / more affordable
- non-GM is cheaper overall / to grow.

In one region of India, Andhra Pradesh, a severe drought in 2002–2003 meant that Bt cotton grew less well than other varieties of cotton that were better adapted for the conditions. Suggest how a variety of Bt cotton that is better adapted to dry conditions could be produced from the existing varieties of Bt cotton, without using gene technology.

- selective breeding / artificial selection.
- cross Bt cotton with a Bt variety that grows well in dry / drought.
- select / choose offspring with Bt trait / gene and grow well in dry / drought.
- repeat crossing / selection for several generations.