Module 6: Genetic Change

Mutation
IQ1: How does a mutation introduce new alleles into a population?

★ Mutation
- Mutation = change in the base sequence of DNA
  - Occur in somatic cells (body) or germ line cells (sex)
  - May occur spontaneously or be induced by mutagens
  - New alleles can be formed in a population
- Mutagen = environmental agent that causes a genetic mutation
  - Physical, chemical or biological

- Physical: Electromagnetic radiation sources
- Radiation is highly penetrative - composed of high-energy waves
- It is able to enter cells from external sources and interfere with DNA molecules in the nucleus
- Interference by electromagnetic radiation can cause hydrogen bonds within the DNA structure to break - may change the chemical composition of the DNA molecule
- Leads to a mutation if the DNA repair system is unable to fix the change or repairs it incorrectly
- Eg. UV light breaks the hydrogen bond between nitrogenous bases in the DNA molecules

- Chemical mutagens
- Radioactive agents eg. uranium release radiation in the form of alpha and beta particles
  - Able to penetrate the cell and interfere with DNA bonding to create disruptions
- Intercalating agents eg. ethidium bromide - chemicals that insert themselves into the bonds between base pairs and alter the shape of the DNA, leading to errors in replication
  - Can cause frameshift mutations during DNA replication
- Metals - affect processes in DNA repair such as the ability for proteins to recognise base-pair mismatching
  - Affects cell's ability to correct errors, allowing mutations to proliferate
  - Reduce fidelity during DNA replication - cell does not correctly copy the genetic code when it is replicating - errors are incorporated into the new DNA strands
  - Eg. Nickel - inhibits the ability of histones to condense DNA, affecting chromosome mutation

- Naturally occurring mutagens

Biological Mutagens	Non-biological mutagens
• Viruses insert their genome into a host cell and can disrupt the host DNA • Bacteria infections can induce	• Natural sources of radiation include cosmic radiation from the sun and radiation in rocks and soils

inflammation which may reduce the efficiency of DNA repair systems, increasing the rate of mutation ● Fungi produce mycotoxins that can be mutagenic to other species	● Natural sources of chemical mutagens include chemicals produced by incomplete burning eg. bushfire and chemicals that occur naturally in the environment eg. mercury

★ Comparing the causes, processes and effects of mutations

- Point mutations

- Change of one or more nucleotide bases in DNA
- Occurs during replication before mitosis or meiosis
- May result from exposure to mutagens
- Include: base substitution, frameshift mutation

Base substitutions	● One base being replaced with an incorrect base in a DNA sequence ● Types of base substitution: ○ Silent - altered bases code for the same amino acid ○ Missense - change in base gives rise to a new amino acid ○ Nonsense - change in base causes the creation of a stop codon ○ Neutral - an amino acid of the same type as the original - change does not affect the structure of the protein
Frameshift mutation	● One or two nucleotides being either added or removed from a nucleotide sequence ● As every codon is altered, every amino acid they code for after the point of mutation is altered ● Results in the loss of a functional protein - likely that the resulting polypeptide would be completely different ● In cases where the frameshift mutation creates a stop codon earlier in the sequence, the resulting polypeptide will be shorter and would negatively impact cell functioning ● Types of frameshift mutation: ○ Insertion - nucleotides are added into a sequence ○ Deletion - nucleotides are deleted from a sequence

- Chromosomal mutations

- Changes to the entire chromosome or significant parts of the chromosome
- Result from problems in chromosome behaviour during meiosis, mitosis or mutagens

<table>
<tr><td>Structural abnormalities</td><td>Block mutation - a permanent change to a segment of a chromosome that rearranges, deletes or disrupts many loci
Occur during meiosis in eukaryotic cells
Can be caused by mutagens such as radiation
Types of block mutations:
Duplication - section of a chromosome is doubled
Deletion - removal of sections of a chromosome
Inversion - section of a chromosome is turned upside down and re-inserted into the chromosome
Translocation - section of one chromosome moves to a non-homologous chromosomes</td></tr>
<tr><td>Numerical abnormalities</td><td>Chromosome abnormality - when a mutation involves whole chromosomes or the number of chromosomes
Chromosome number:
Trisomy - extra chromosome
Deletion - missing chromosome
Polyploidy - extra complete set of chromosomes
Main forms of chromosome abnormalities
Aneuploidy = addition of all or parts of a chromosome eg. Down Syndrome
Polyploidy = possession of more than two sets of chromosomes per nucleus
Can be caused by:
Non-disjunction - error during mitosis or meiosis when both members of a pair of chromosomes or both sister chromatids fail to separate properly
Unequal crossing over in meiosis - crossing over between adjacent non-sister chromatids is unequal - one cell may have a chromosome with a gene deletion and another cell with a gene duplication</td></tr>
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★ Effects of somatic and germ-line mutations on organisms

- If the mutation occurs earlier in development - has greater effect
  - Mutation will be replicated in a greater number of cells as the organism grows

	Somatic mutation	Germ-line mutation
Location	• Somatic cells	• Gametes
Causes	• Occurs due to replication errors prior to mitosis	• Same as somatic

Effect on offspring	- Not passed onto offspring - Mutations only affect the cells that are produced by the mutated cell - Earlier a mutation occurs, greater its effect will be on an organism's phenotype	Passed onto offspring - as the embryo forms, mutation is replicated via mitosis in every cell of the embryo, affecting all cells in the resulting offspring
Example	Eg. a mutation of the tumour suppressor genes eg. BRCA1 - could lead to cancer but won't be passed onto offspring	Down Syndrome - during meiosis, chromosomes fail to separate properly, resulting in one gamete receiving an extra chromosome 21 while the other receives no copy

★ Coding and non-coding DNA segments

	Coding DNA (Exons)	Non-coding DNA (Introns)
Definition	- Sequences of DNA that code for a protein - Have been transcribed and translated	- Sequences of DNA which do not code for a protein - Many code for end-products other than DNA (eg rNA) - Junk DNA = parts of non-coding DNA that are not involved in gene regulation - Regulating DNA sequences: - Promoter = section of a gene that is **found on the DNA before the start codon** - located where RNA polymerase attaches to the gene - Enhances= regions of the DNA where **transcription factors bind and increases the rate of transcription** - Silences = regions of DNA where transcription factors

		◦ bind and repress the expression of the gene they control ◦ Terminators = section of DNA marking the end of a gene - trigger the release of the completed mRNA construct
Consequences of mutation	• Can have significant effects on protein (eg. point mutation) or no impact (eg. silent mutation)	• Have no effect on an organism
Example		• Eg. Huntington's disease • Mutation in the Huntington gene on chromosome 4 • More than 40 tandem repeats of the sequence CAG results in the disease.

★ Causes of genetic variation: fertilisation, meiosis and mutation

	Fertilisation	Meiosis	Mutation
Definition	• Occurs during sexual reproduction when 2 gametes combine to form a zygote • Full set of chromosomes is restored (23 pairs, 46 in total)	• Production of gametes • One parent cell becomes 4 daughter cells, each with half the number of required chromosomes	• Change in the base sequence of DNA • Somatic or germline mutation introduce new alleles
Causes	• Interaction of dominant and recessive genes from chromosomes	• DNA replication - random mutations may be introduced	• DNA replication error during Interphase I meiosis that are

	of 2 different parent organisms	as a result of replication error • Crossing over - homologous chromosomes line up and exchange segments of DNA to produce new gene combinations within sister chromatids • Random segregation - alleles separate randomly from one another during gamete formation • Independent assortment - alleles for different traits are sorted into gametes	NOT repaired or corrected by repair enzymes • Induced mutation - exposed to mutagens and radiation

★ Effect of mutation, gene flow and genetic drift on the gene pool of populations

- Gene pool = total number of genes of every individual in a population
- Natural selection = one allele (or combination of alleles of different genes) makes an organism more or less likely to survive and reproduce in a given environment
- If an allele reduces "fitness", its frequency will tend to drop from one generation to the next
- Advantageous alleles that make individuals suited in the environment will ↑ over generations

	Mutation	Gene flow	Genetic drift
Effect	• New genes arise due to 'errors' in DNA replication during meiosis • May be beneficial, neutral or harmful • May improve survival and	• Gene flow = movement of genes into or out of a population due to either the movement of individual organisms or their	• Genetic drift = changes in allele frequency due to chance events • Does not take into account an allele's benefit or harm to

	reproductive ability - ↑ in allele frequency with each generation	gametes between populations
		• Allele frequency in the population changes
		  ○ Some alleles lost due to emigration
		  ○ Alleles gain to immigration
			the individual that carries it
			• Two causes of genetic drift: bottleneck and the founder effect

Causes of genetic drift:		
	Bottlenecking	Founder effect
Definition	• Decrease in the number of individuals in a population due to a natural disaster • Non-selective - individuals that survive are due to chance and not the presence of advantageous traits	• Small proportion of individuals (founding individuals) break off and become isolated from the larger population (original population) to establish a new colony
Effects	• Genetic variation ↓ as number of mating individuals will ↓ - only some individuals survive and this will affect the surviving population for following generations	• May lead to new speciation events and evolutionary pathways
Examples	• Eg. Cheetahs - cheetah population has little genetic diversity • They have escaped extinction at the end of the last ice age • Lack of genetic variation has led to sperm abnormalities, vulnerability to disease, etc	• Eg. fungi release spores which colonise in different environments, forming new colonies that may not have the same alleles as the population from which they were originated

Biotechnology

IQ2: How do genetic techniques affect Earth's biodiversity?

★ Uses and applications of biotechnology (past, present and future)

- Biotechnology = the manipulation of living organisms or biological processes for human benefit

Past	Present	Future
• Food production - use of living cells to make bread, cheese and wine • Selective breeding - selecting seeds from the best crops and breeding the best-quality animals	• Antibiotics - medicine that inhibits the growth of bacteria • Artificial insemination - reproductive technology used to select favourable traits to be passed onto offspring • Cloning - producing genetically identical individuals of an organism either naturally or artificially • GMOs/transgenic species - Bt cotton	Gene editing (CRISPR) • Genes can be spliced and inserted with accuracy eg. uncovering genes that cause neurological disorders • Easy use and accuracy of CRISPR raises concerns about germline gene editing and creation of 'designer babies' Edible vaccines • Vaccine antigens are protected by the cell wall of the plant cells and once ingested, are released in the intestines and absorbed into the circulatory system • Ideal for medicine in rural communities and less developed countries • Low cost alternative to regular vaccine

★ Social implications and ethical uses of biotechnology, including plant and animal examples

	Plant - Bt Cotton	Animal - Antithrombin III in goats

Description	• Transgenic crop that has been genetically modified to contain two genes from the soil bacterium, Bacillus thuringiensis • Expression of these genes produces proteins in the cotton plant that kill the main caterpillar pest of cotton by disrupting its digestive system	• Genetically engineered female goat can produce antithrombin III in their milk Process: • DNA for human antithrombin is injected into the fertilised goat cells • These cells develop into embryos, which are implanted into surrogate mothers
Social (Medical and health)	• Farmer's become dependent on companies that patents seeds with transgenic properties ○ As Bt cotton seeds lose their efficacy after one generation, farmers are forced to purchase new seeds each year, limiting their income • Long term effect on food consumption - caterpillar population declines, affecting the food chain as there is less food for predators and the pathogens that attack them	• Blood donations are often in short supply so there is not enough blood to treat antithrombin III deficiency ○ Less human blood donations are required
Ethical (Moral, religious and legal)	• Debate whether a company has the right of ownership over a living thing or biological property eg Bt ○ Should a company be allowed to monopolise control over Bt where they can set high prices and disadvantage poorer farmers, especially developing countries	• Quality of life may be impaired by genetic modification techniques or in the transgenic production facility ○ Procedure may be painful for the animal and may result in mental and physical trauma of the animal

★ Future directions of the use of biotechnology

★ Potential benefits for society of research using genetic technologies

	CRISPR	Edible vaccines in plants

Description	• Gene editing system where point mutations are accurately introduced to genomes
	• Composed of 2 parts:
	   ○ Enzyme Cas-9: unzips target DNA
	   ○ Guide RNA: directs enzyme to location where to cut DNA
		• Vaccine antigens are protected by the cell wall of the plant cells and once ingested, are released in the intestines and absorbed into the circulatory system

Benefits	• Genes can be spliced and inserted with accuracy eg. uncovering genes that cause neurological disorders • Has the potential to improve targeted gene therapy, reversing point mutations which cause diseases such as cystic fibrosis • Scientists can edit mutant sequence and 'knock in' a gene for cystic fibrosis • Scientists can 'knock out' genes that cause Alzheimer's Diseases	• As they do not require refrigeration - ideal for medicine in rural communities and less developed countries where access to suitable transport is not always reliable • Low cost alternative to regular vaccine
Concerns	• If used in germline cells or embryo, it can be passed onto future generations • Raises concerns about germline gene editing and creation of 'designer babies'	

★ Changes to the Earth's biodiversity due to genetic techniques

- Biodiversity = variety of life on Earth including plants, animals and microorganisms

Short term changes	• ↑ biodiversity by introducing new gene combinations
Long term changes	• ↓ biodiversity by selectively breeding desired gene combinations • Large numbers of identical organisms with particular characteristics are created • Putting population at risk of extinction through disease or sudden environmental change

Genetic technologies

IQ2: Does artificial manipulation of DNA have the potential to change populations forever?

★ Uses and advantages of current genetic technologies that induce genetic change

Methods		Uses	Advantages
Reproductive technologies	Artificial insemination	Collecting sperm from a chosen male with desirable characteristics and artificially introducing it into several selected females	
	Artificial pollination		
Cloning techniques	Whole organism cloning		
	Gene cloning		
Recombinant DNA techniques	Transgenic species		
	Gene therapy		
	CRISPR		

★ Compare the processes and outcomes of reproductive technologies

- Reproductive technologies

- Reproductive technologies = technology that is used to bring about reproduction or increase the breeding success of an individual
- Aim - pass on desirable characteristics to the next generation

	Artificial insemination	Artificial pollination	In vitro fertilisation (IVF)
Description	• Collecting sperm from a chosen male and artificially introducing it into several selected females	• Involves taking the pollen from one plant and placing it on the stigma of another flower	• Process in which an egg is fertilised by sperm outside the body
Processes	1) Semen containing the sperm is extracted from the male using mechanical stimulation or an artificial vagina 2) Extracted semen is divided into semen straws, then frozen in liquid nitrogen for long-term storage 3) Semen straw is placed in a artificial insemination 'gun' 4) Gun is inserted into uterus of female	1) Pollen (sperm) is removed from the stamen of one plant 2) Pollen applied to the stigma of another plant 3) Pollen fertilises the ovum	1) Hormone treatment to stimulate egg production 2) Removal of multiple eggs from ovaries 3) Fertilisation (eggs and sperm are combined in the lab) 4) Incubation - leading to the production of embryos 5) Embryos are implanted into the uterus or frozen
Adv	Positives • Favourable genes passed to offspring • Short-term - ↑ biodiversity • Semen samples are screened for disease -	Positives • Higher crop yields • Selection of desirable traits • Creation of new plant species	Positives • Favourable genes passed to offspring • Allows for genetic screening of embryos to avoid disease

	improves reproductive health of the population ● Transporting frozen sperm - cost-effective, reduces danger of animals being injured in transmit or during mating		
Disadv	Negatives ● Long-term - ↓ biodiversity ● Desirable genes from the same sperm donor is used to inseminate many females - all offspring have the same genes ● Problems if recessive characteristics show up more frequently in the phenotype ● Specialised equipment and high quality sperm can be costly	Negatives ● Loss of biodiversity ● Overuse can lead to crops that are too similar - ↓ biodiversity ● Creation of monocultures	Negatives ● Expensive ● Genetic diversity ↓ - production of large numbers of viable embryos from a small selection of parent animals with desirable traits ● Sperm banks can alter genetic composition of a population - ↑ the frequency of favourable donors

★ Effectiveness of cloning

- Clone = organism that has the identical genetic code of the parent cell
- All asexual forms of reproduction produces clones
- Two main forms of cloning:
  - Gene cloning = producing identical copies of one gene
  - Whole organism cloning = producing a genetically identical organism

- Whole organism cloning

- Whole organism cloning = single cell is used to make an entire organism through recombinant DNA technology

- Techniques: somatic cell nuclear transfer (SCNT), artificial embryo twinning

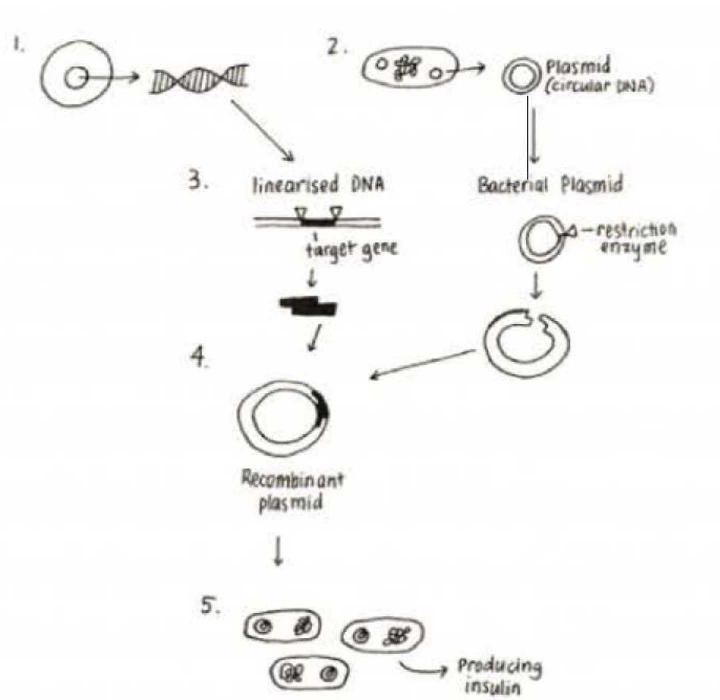
	Somatic cell nuclear transfer (SCNT)	Artificial embryo twinning
Description	• Involves 3 animals: donates the nucleus, egg donor and surrogate mother	• Process that splits an embryo at an early stage of development and mimics the natural process of producing identical twins
Process	1) Adult sheep tissue cell (mammary gland cell) is removed from sheep and cultured in the lab. The cells are placed in a special media to stop the cell cycle and arrest cell division 2) Using a micropipette, the nucleus is removed from one of these adult cells and injected into an enucleated egg cell (egg cell with genetic info - nucleus - removed) from a second sheep 3) Gentle electric pulse causes nucleus to fuse with egg cell 4) A second electric pulse starts cell division and embryo formation 5) Embryo is implanted into a female sheep (surrogate) where it grows into a new organism	• Egg is artificially fertilised by a sperm and a zygote forms • Unspecialised cells of the resulting embryo are then separated during the first few days of growth • Creates multiple identical embryos which are each implanted into the uterus of a surrogate mother to grow and develop • Resulting offspring are genetically identical to each other

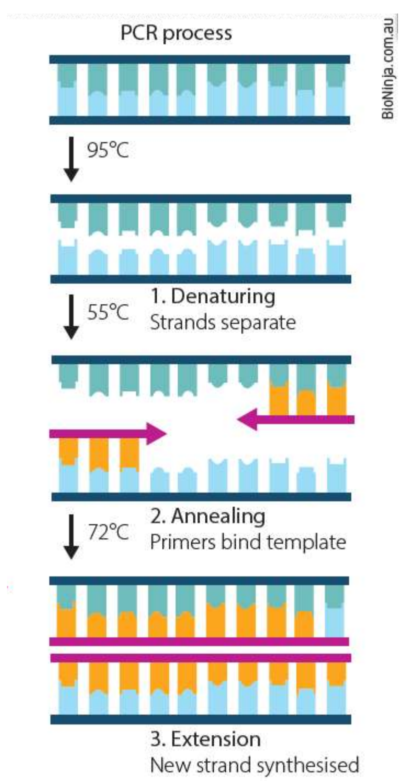
- Gene cloning

- Gene cloning = used to produce identical copies of a specific gene
- Can be used to produce many different proteins from many different organisms
    - Also can combine different proteins recombinantly
- Can occur via two techniques:
    - In vivo - within a living organism
    - In vitro - outside a living organism

	In vivo	In vitro (PCR)
Process	1) A useful target gene is identified	• PCR can be used to amplify the gene of interest

	2) Plasmids are isolated from bacteria 3) Both the bacterial plasmid and the DNA containing the target gene are treated using a restriction enzyme. Restriction enzymes 'cut' DNA, breaking hydrogen bonds in the molecule. These enzymes create 'sticky ends', sequences of overhanging single stranded DNA 4) The complementary sticky ends of the target gene and the plasmid come together through base-pairing affinity - recombined using DNA ligase 5) The new recombinant plasmid is re-inserted into host bacteria by a process called transformation 6) The host cell can now make copies of the vector DNA each time the cell divides	● Involves a process of thermal cycling to denature the DNA strand and the use of contemporary primers that locate and duplicate the required section of DNA Process: 1) Denaturing - DNA is heated to break the hydrogen bonds between the two strands, exposing the nitrogen bases 2) Annealing - the mixture is cooled to allow the primers to bind to the exposed template strands 3) Extension - the temperature is raised moderately to allow DNA polymerase to attach nucleotides to the template strand starting from the primers 4) Multiple cycles are repeated to allow the target sequence to be copied many times





★ Techniques and applications used in recombinant DNA technology

- Recombinant DNA technology = joining together of DNA molecules from two different species to give a transgenic species

- Forming recombinant DNA and gene cloning

1) A useful gene is identified and isolated from a cell using restriction enzymes
2) A plasmid is removed from bacteria and cut with the same restriction enzyme, producing the same sticky ends (single stranded overhangs) as the isolated gene
3) The fragments of DNa (plasmid and gene of interest) are allowed to recombine via base pairing due to their matching sticky ends - joining is facilitate by DNA ligase
4) The recombinant plasmid is inserted back into a bacterial cell where multiple copies of the gene can be produced

- Delivering the gene into the organism to be genetically modified

- The gene must become part of the organism's germline genome if it is to be inherited by subsequent generations
- Gene is inserted directly into a germline cell or into a fertilised egg cell

1) Microinjection of DNA through a fine glass needle into the pronuclei of a fertilised egg cell of another species

- After fusion of the pronuclei, the gene becomes part of the DNA of the newly formed organism

2) Biolistics

- DNA on microscopic particles are mechanically delivered into target tissues and cells by firing them from a gene gun
- Eg. tiny gold particles are used to coat DNA which is then fired at target cells under high pressure or voltage

3) Electroporation

- An electric current is applied to the cell to increase its permeability to take up the recombinant DNA

4) Viral vector

- Recombinant DNA is carried into cells via a viral vector
- Viruses are naturally capable of inserting their genetic information into their host cells
- Viral vectors may be injected or inhaled

★ Benefits of genetic technologies in agricultural, medical and industrial applications

	Medical - Antithrombin III in goats	Agricultural - Bt Cotton	Industrial - Spider silk in transgenic bacteria

Description	• Genetically engineered female goat can produce antithrombin III in their milk Process: • DNA for human antithrombin is injected into the fertilised goat cells • These cells develop into embryos, which are implanted into surrogate mothers	• Transgenic crop that has been genetically modified to contain genes from the soil bacterium, Bacillus thuringiensis • It allows the plant to produce a protein which makes it pest-resistant	• Genetically modified E.coli produce fibre filaments • The genes of silk glands of spiders are cloned and inserted into the genome of E.coli
Adv	• Prevents blood clots and treat diseases that cause AT-III deficiency • Lower cost pharmaceutical in greater quantity	• Save money and time (economic) • Reduces use of insecticides which benefits food chain, web and human health (environmental)	
Disadv	• Quality of life may be impaired by genetic modification techniques or in the transgenic production facility ○ Procedure may be painful for the animal and may result in mental and physical trauma of the animal	• Farmers have to buy the seeds each year and are dependent on large companies (only farmers with lots of money can access) • Reduces biodiversity as large areas of crops are genetically identical • Insects could become resistant to Bt chemicals	• Requires large vats to grow bacteria and machinery to purify/harvest the silk proteins

★ Effect on biodiversity using biotechnology in agriculture

	Bt Cotton	Artificial insemination
Positive (+)		• Allows crosses that would not normally occur due to distance or physical differences
Negative (-)	• Potential loss of original genes - pests can develop resistance • Widespread monoculture reduces ability of farmers to react to changes in the environment eg. new pests - whole crops could be wiped out if a disease that attacks the specific gene emerges • Reduce the food available for species that feed on them, affecting food chains and food webs, thus reducing the number of species that can survive • Reduce biodiversity of caterpillar population - susceptible varieties are killed and only those that are resistant survive to reproduce • Large crops are genetically identical - may outcompete native species, reducing ecosystem biodiversity	• One male has many offspring - all offspring will be genetically half siblings - reduces genetic variation

- Impact of scientific knowledge on the manipulation of plant and animal reproduction

- Agriculture - cultivation and breeding of animals, plants and fungi for food and other products

- Hybridisation and genetic diversity

- In selective breeding, humans choose desirable traits like disease resistance, high yield, fast growth to maturity to create stain or hybrids that are used to produce the next generation
- If wild varieties are not kept or allowed to reproduce, genetic variability will decrease

- In an ecosystem, genetic biodiversity is needed for sustained functionality and stability
- Agricultural biodiversity is directly affected by the choices made by farmers on which species, varieties, breeds that they will use and farm from year to year
- Agrobiodiversity is decreasing as world food supplies rely on fewer and fewer plant and animal species
- The need to produce higher yielding crops with specific features means that many original crop plants have been lost as fewer varieties are used each year
- To conserve biodiversity, seed banks have been established
- The loss of pollinators, especially bees has affected biodiversity
- Many plants rely on pollinators to enable fertile seed production and many crops eg. apples, squash and almonds will not produce fruit and seeds without pollination

★ Influence of social, economic and cultural contexts on a range of biotechnologies

Social	
Economic	
Cultural	