Selective breeding of organisms with favourable characteristics is an early example of biotechnology. Increased knowledge of molecular biology and inheritance has revolutionised biotechnology and has benefits for medicine, agriculture, the environment among other industries.

**Gene technology** or **genetic engineering** is where techniques are applied to manipulate the functioning of genes to suit the needs of society in a precise way, for example switching genes on or off, remove or insert genes (pesticide, protein production for human use), use bacteria with inserted human genes to build proteins for human use etc.

**Tools and techniques**
Used for determining function of genes, transferring genes, screening for alleles, DNA profiling cloning. Tools need to cut, synthesise and paste DNA into cells, and view an analyse DNA.

**Cutting DNA**
Restriction enzymes (restriction endonucleases) cut DNA in restriction fragments at specific DNA sequences called recognition sites. Different enzymes have different recognition sites. These enzymes are extracted from bacteria, each recognising a sequence of 4 – 8 nucleotide base pairs where they bind and cut the DNA. Cut ends could be uneven producing sticky ends or even producing blunt ends.

**Recombining DNA**
DNA ligases are enzymes that join 2 pieces of DNA together. Can join 2 strands with exposed complimentary nucleotides by re-establishing the H bond b/w nucleotides. 2 DNA pieces cut with the same enzyme will have the same sticky ends so can be recombined – recombinant DNA technology.

**Amplifying DNA – polymerase chain reaction (PCR)**
PCR uses the enzyme DNA polymerase to catalyse the formation of new DNA molecules. This amplifies or makes many copies of the specific DNA required for study.

A thermic cycler carries out the process and requires a number of components:

- The DNA to be copied
- DNA polymerase
- Buffer solution containing salts & other chemicals that allow the polymerase to function
- A supply of the 4 nucleotides found in DNA to produce the new DNA
- 2 primer sequences, single DNA strands complimentary to the sequences at each end of the DNA section being copied, - starting point for polymerase to act at.

**Steps in PCR cycle**
1. **Denaturation**: double DNA strand heated to 95°C breaking the H bonds, producing 2 strands
2. **Attachment of primer**: Temp dropped to 50-55°C to allow base pairing and H bonds to form so primers can join to opposite ends of the strand.
3. **Extension**: temp raised to 72°C and new DNA strands are synthesised using DNA polymerase and available nucleotides.

Cycle repeated until enough DNA is obtained. 20 cycles will produce over 1 million copies.

**Q 3, 4, 5**
Biozone: 213 What is GM, 214 Applications, 215/6 Restriction enzymes, 217 Ligation, 231 (just read)
Using recombinant bacteria, 233 production of human proteins

**Q 6, 7, 9**
Biozone -219/20 Polymerase chain reaction
Sorting DNA fragments – gel electrophoresis

Gel electrophoresis – a technique that separates fragments of DNA according to size and charge.

Phosphate groups in DNA give an overall negative charge gel electrophoresis makes use of this.
1. DNA samples placed in wells at negative end of a piece of agar gel in a tray.
2. Positive & negative electrodes are placed at each end of the gel.
3. When current runs fragments are repelled from -ive end, move towards +ive electrode. Smaller fragments travel faster than large ones. DNA not visible in gel.
4. DNA sample mixed with loading dye that attaches DNA to before loading into gel.
5. Photograph (autoradiograph) taken in fluorescent light that shows up the dye
6. Distance move by fragments depends on their size. Molecular markers are pieces of DNA with a known number of bases – used to determine size of separated DNA fragments.

Probing for genes

A gene probe searches for a particular sequence of DNA by binding to the target sequence. Useful in locating alleles that may cause disease. The gene probe is a single DNA strand that has complimentary bases to the target gene. DNA being investigated is heated to separate the strands then the probe is introduced and will bind with complimentary strands. The probe has a radioactive tag or fluorescent dye attached to enable them to be found.

DNA sequencing

DNA sequencing is a process where the exact nucleotide sequence of a gene is determined. Can be done manually using gel electrophoresis, or automatically by machines. The 4 nucleotides are labelled with 4 different fluorescent dyes. Only 1 copy of the DNA is required, a computer analyses the data.

The Human genome project mapped the sequence of the whole human genome.

Q 11, 12, 13, 14, Biobox 9.3 and 9.4

Biozone – 218 – gel electrophoresis, 241 – Automated DNA sequencing

Gene cloning

If functioning copies of genes are required they need to be produced in a living organism. Bacteria have the gene of interest inserted into a plasmid that reproduces resulting in clones of the gene.
1. Plasmid removed from bacteria
2. Same restriction enzyme cuts plasmid and DNA to be inserted to give complimentary sticky ends
3. DNA ligase binds foreign gene into plasmid to produce the recombinant plasmid.
4. Recombinant plasmids are added to bacterial culture and are taken into some bacteria (transformation)- these are isolated. The plasmids replicate producing multiple copies of the foreign gene.
5. The gene can be studied or used to produce useful human proteins.

Transferring genes

Transferring genes is the basis of gene therapy. Functional genes are inserted to produce a required protein and alleviate symptoms of a disease. Gene needs to be inserted and be functional in host organism. A vector (plasmid, liposome or virus) has the gene inserted and carries it into the host organism. When foreign DNA has been inserted the organism is transgenic or genetically modified.

Viral vectors -desired genes inserted into viral DNA or RNA and disease causing genes removed or disabled, virus infects target cell. Immune system may attack these and decrease chance of survival.

Liposome vectors – small circular molecules surrounded by plasma membrane, which fuses with other membranes delivering contents into cell. Can be made artificially with inserted genes- target specific cells

Plasmid vectors – recombinant plasmid inserted into organism but DNA is unstable in body cells.

Questions 15, 16, 17, 18, 19, 20, Biozone – 225/6 gene cloning plasmids, 237/8 gene delivery systems
**DNA profiling (genetic or DNA fingerprinting)**
Process used to compare base sequence of individuals. DNA in families is more similar than in strangers.

**Short tandem repeats (STRs)** section of non-coding DNA 2 – 5 bases long repeated many times

**Variable nucleotide tandem repeats (VNTRs)** section of non-coding DNA more than 5 bases long repeated many times

**DNA profiling** identifies people based on unique differences in the length of their DNA repeats.

**Steps involved in DNA profiling**
1. DNA sample obtained from cell of the individual
2. DNA extracted
3. PCR amplifies the DNA of up to 13 different STR regions
4. Gel electrophoresis sorts DNA fragments according to length
5. Samples compared

Profiling is useful in forensic investigations, paternity cases, identification of unidentified individuals.

Biobox 9.5 & 9.6, Questions 22, 23

**Biozone -221 – DNA profiling using PCR,**

**Applying biotechnology**

**Gene technology & cancer** - Cancer arises from a mutation that causes uncontrolled cell division- these cells can metastasise or spread to other tissues and cause cancer there. Disrupted genes responsible can be identified in some tumours, allowing more accurate diagnosis of the type of tumour and determination of most effective drugs. Most cancers are sporadic but some families have a history of some cancers. Family members are tested regularly - diagnosed or treated earlier. **Gene therapy** could result in cancerous cells being injected with genes that produce toxins in cells where cancer genes are active thus killing them.

**Malnutrition –crop modification** (inserting foreign genes into plants) can produce crops with higher yields, better nutrition, pest resistance, drought tolerance, introduced nutrients or vaccines.

**Medicine – applications include**

**Genetic testing** – examining DNA or products of gene action to determine if someone has an allele of a gene associated with a disorder. Identifies carriers of a gene mutation or potential sufferers of a disorder.

**Pharmaceuticals produced by gene cloning** involve inserting a target gene (eg for insulin or growth hormone production) into a plasmid that is taken up by bacteria, resulting in many bacteria producing large amounts of the required protein which can be used for therapeutical and commercial purposes. Yeasts have produced vaccines, other mammals have produced other human proteins.

**Gene therapy (somatic cells)**- the insertion of fully functional genes into an individual suffering from a disorder caused by a mal-functioning gene. A vector is used to deliver genes to the affected areas. Bone marrow cells from individuals with a gene disrupting B and T cell function had viral cells introduce a functioning version of the gene, and were then reintroduced to the bone marrow resulting in production of functional B and T cells. These altered cells cannot be passed onto offspring, so disorder can still be passed on. There are also still problems delivering the genes to cells where they are required.

Questions 24, 25, 26, 27, 28, 30, 31, Biozone – 235 – gene therapy

**Agricultural & Environmental applications**

**Transgenic crops and livestock** can be developed with pesticides (reduces chemical spraying), herbicide resistance (reduces plant damage from herbicides), increased yields and other desirable features.

**Conservation** – DNA testing can determine how similar individuals in a population are and if too little variation exists, breeding programs can increase variation & increase possible resistance to disease.

**Pest eradication** could reduce spread of diseases such as malaria. An inserted gene that produces a restriction enzyme to interfere with the development of a fertilised egg could be passed on through generations of mosquitos and could greatly reduce their numbers.
**Emerging areas of biotechnology**

**Cloning** – making identical copies of an original. (eg. genes, cells, organisms)

**Embryo splitting** – eggs are removed from donor, fertilised by donor sperm in vitro, zygote is allowed to divide then the cells are separated and implanted into different surrogate mothers to develop into 2 genetically identical individuals.

**Nuclear transfer** resulted in Dolly the sheep. The nucleus from a recipient egg is removed and a somatic cell from a mature donor animal (with nucleus in tact) is inserted into the egg. The blastocyst that develops is implanted into a surrogate mother to develop. The offspring will be genetically identical to the mature donor animal, not the egg supplier nor the surrogate mother.

**Stem cell technology**
Stem cells are unspecialised and can develop into any type of cell and have the capacity to keep dividing. They have the potential to replaced damaged tissue such as in spinal injury, burns, heart or Parkinson’s disease and diabetes. They could be used to test drugs or affect of other chemicals.

**Embryonic** stem cells come from 4-5 day old embryos and can form any type of cell but require the destruction of the embryo, raising ethical issues. **Adult** stem cells can produce only limited range of cells but may be safer to use and raise fewer ethical issues. New research is examining the use of stem cells from the blood of the umbilical cord.

**Questions 34, 35, 36, 37, 38, 39**
**Biozone** – 227/8 transgenic organisms, 229/30 (just read) GM plants, 232 edible vaccines, 248 cloning – embryo splitting, 249/50 cloning - nuclear transfer, 253/4 stem cells

**Implications and issues**
**Genetically modified organisms (GMOs)** have great advantages but raise questions about their effect on the environment and on other organisms that come into contact with them.

What are the side effects of genetically modified foods?
Will allergies increase?
Is it ethical to combine genes from different organisms?
Will they lead to the use of more herbicides or result in herbicide resistant weeds?

**Medical issues**
Is gene therapy safe?
What are the long term effects?
Should insurance companies have access to the results of genetic testing?
Who should pay? Will gene therapy be only available for those who can afford it?
Should family members have access to test results?
Should companies be able to take out patents to stop other companies researching for cures?

**Questions 41, 42, 43**
**Biozone** - 239 Ethics of GMS technology

**Apply understanding 1, 2, 3, 5, 6, 7**