# Edexcel (B) Biology A-level

## Topic 7: Modern Genetics

Notes

# Using Gene Sequencing

**Genome** = all of an organism's DNA, including mitochondrial/chloroplast DNA.

**Polymerase chain reaction (PCR)** is used to amplify the DNA by making millions of copies of a given DNA sample. It occurs as following:

1. A reaction mixture is set up by mixing the **DNA sample, primers, free nucleotides and heat-stable DNA polymerase,** which is the enzyme involved in creating new DNA strands.
2. The mixture is then **heated to 95 degrees Celcius** to break the hydrogen bonds between the complementary bases and to separate the two strands.
3. The mixture is then **cooled to a temperature between 50-65 degrees**, depending on the type of primers used, so the primers can bind to the strands (annealing).
4. Temperature is increased to about **70 degrees,** as this is the temperature DNA polymerase works at. DNA polymerase creates a copy of the sample by complementary base pairing using the free nucleotides.
5. **This cycle is repeated around 30 times** and gives rise to an amount of DNA sufficient to create a DNA profile.

Amplified samples can be used in **DNA sequencing** and **DNA profiling**.

DNA sequencing is used to predict the amino acid sequence of proteins and determine possible links to genetically determined conditions:

- The DNA sample is divided into **four separate sequencing reactions** which contain all four **standard nucleotides, DNA polymerase, primers required for replication and terminator nucleotides** which have been **fluorescently labelled** for ease of identification.
- When a terminator nucleotide is incorporated into a growing chain, replication is terminated.
- DNA fragments of different lengths are produced across the reaction vessels.
- **High-resolution gel electrophoresis** is used to separate the fragments by size – single base differences can be seen.
- The fragments are visualised under **UV light**, thus enabling the base sequence to be read from the bottom of the gel upwards.

The rapid advancement of techniques used in sequencing increased the speed of sequencing and allowed whole-genome sequencing.

**DNA profiling** is a forensic technique used to **identify criminals and test paternity:**

1. Fragments of DNA are cut with **restriction endonuclease enzymes** (either side of satellites).
2. These fragments are separated and visualised using **gel electrophoresis** - fragments are placed in wells in agarose gels and dyed with ethidium bromide so they fluoresce under UV light. A current is then applied to the gel. DNA is negative and fragments of different sizes move at different speeds according to mass so 'bands' appear.
3. **Southern Blot** - alkaline buffer solution added, nylon filter - dry absorbent material draws solution containing DNA fragments to the filter - fragments visible as 'blots'. Gene probes (labelled complementary sequences that fluoresce or are radioactive) are added and bind with DNA (hybridisation).
4. 'Blots' compared and the number of satellites visualised as a graph (repeated sequences of DNA in introns are referred to as **mini/microsatellites** depending on their size. The more closely related two people/species are, the **more similar the repeats** are).

# Factors Affecting Gene Expression

**Transcription Factors**

**Transcription factors** are proteins that bind to DNA.

Transcription factors bind to specific base sequences:

- **Promoter** Sequences
  - Found upstream of the gene they act on - enable the binding of RNA polymerase and therefore promote transcription.
- **Enhancer** Sequences
  - Regulate DNA activity by changing chromatin structure - making it more or less open to RNA polymerase. Open = active gene expression, closed = gene inactivity - transcription factors either stimulate or prevent transcription of the gene.

**Epigenetics** = heritable and reversible modifications to the DNA that do not involve changes to the nucleotide sequence

**DNA Methylation**: addition of a methyl ($CH_3$) group to a CpG site (cytosine next to guanine) on DNA. Prevents transcription and affects histone structure to make more/less DNA accessible to RNA polymerase.

**Histone Modification**:

1. **Acetylation** - addition of an acetyl ($COCH_3$) group- activates chromatin and allows transcription.
2. **Methylation** - addition of a methyl group- can cause activation/inactivation of chromatin depending on the position of the lysine.

**Non-Coding RNA**: ncRNA affects transcription/modifies the products of transcription e.g. ncRNA coats one X chromosome, which supercoils and condenses to form the stable, inactive Barr body to maintain the balance of gene products.

**RNA splicing**: post-transcriptional modification of mRNA. Eukaryotes produce more proteins than they have genes - RNA splicing explains how, because it results in different products from a single gene.

1. Gene is transcribed which results in pre-mRNA (the transcript of the whole gene).
3. **All introns** (non-coding regions) **and some exons** (coding regions) are **removed**.
4. The remaining genes are joined together by enzyme complexes called **spliceosomes**. The same exons can be joined in a variety of ways to produce several different versions of mature functional RNA.

Epigenetic modifications are important to **ensure cell differentiation**: the process by which a cell becomes specialised for a particular function and produces only its own specific proteins/ 'housekeeping' proteins.

## Stem Cells

**Stem cells are undifferentiated cells** which have the ability to differentiate into many different cell types. Types of stem cells include:

- **Multipotent** cells, which can give rise to multiple types of cells.
- **Pluripotent cells,** which can **give rise to many types of specialised cells,** but not placental cells.
- **Totipotent cells,** which can **give rise to all types of specialised cells,** including placental cells.

**Totipotent cells** only occur for a limited time in a mammalian zygote whereas other types of stem cells such as **pluripotent, multipotent and unipotent cells** are found in mature (somatic) mammals. **Pluripotent stem cells** are commonly used in treating human disorders by **replacing damaged tissue.**

Sources of stem cells include **embryonic stem cells, adult stem cells and fused cells**. Stem cells can be used to **treat a variety of diseases** such as diabetes, multiple sclerosis and Parkinson's disease. They can also be used to **replace damaged tissues** such as nerve tissue in spinal cord injuries. However, there are many **ethical issues** related to the use of stem cells.
Stem cells **could save many lives and improve the quality of life** for many people, however, many people believe it is unethical as **embryos are killed in the process** of stem cell extraction. Moreover, there is a **risk of infection when cells are transplanted** and they could also become **cancerous.**

**Induced Pluripotent Stem Cells** are adult stem cells that have been reprogrammed to become pluripotent again:

1. **Fibroblasts** (connective tissue) taken from skin samples.
5. **Viruses** are used as vectors to introduce **four genes for transcription factors.** These factors activate **specific genes** in adult cells to produce pluripotent cells.
6. Cells behave very similarly to embryonic stem cells - however, their effectiveness long-term is not known and cells show a tendency to become cancerous.

The use of iPS cells **overcomes ethical objections** to the use of embryonic stem cells.

# Gene Technology

**Recombinant DNA** includes DNA from more than one organism.

1. Isolation of the gene: 2 different ways
   - Cut out a section of DNA with the gene of interest with **restriction endonuclease enzyme**. Each restriction endonuclease enzyme cuts at a specific (restricted) site in the DNA sequence. This leaves **'sticky ends'** - staggered complementary exposed bases.
   - Use **reverse transcriptase** enzymes to make DNA from mRNA.
2. Cut plasmid with the same restriction endonuclease enzyme to leave **complementary sticky ends**.
3. Join plasmid and gene with **DNA ligase** to form recombinant DNA.
4. Reincorporate plasmid into host nucleus.

The plasmid is acting as a **vector**. Other vectors can be used (necessary in some plants and animal cells):

- Gene Guns (high-speed metal pellets with DNA - some cells will accept the DNA)
- Viruses
- Liposome Wrapping (DNA wrapped in liposomes - fuse with cell membrane)
- Microinjection (micropipette and micromanipulation)

Effective vectors:

- Target the right cells
- Incorporate the gene into the host genome
- Have no adverse side effects

**Gene markers** are used to show where a foreign gene has been inserted. **Fluorescence** and **antibiotic resistance** combined with **replica plating** can be used as gene markers. Bacteria are transferred from a 'master plate' onto plates with antibiotics using a sterile block - if they don't grow, the gene was inserted successfully and the colonies are still on the 'master plate'.

**Knockout Mice** are mice with one or more genes silenced via the insertion of a similar gene that makes the original gene impossible to read. Can be used to investigate gene function or create animal models of diseases to progress understanding of how the disease may be treated in humans.

**Transgenic plants** are plants which contain genetic material from an unrelated organism.

Genetic modification using ***Agrobacterium tumefaciens***:

1. **Ti plasmid** (transfers bacterial genetic information directly to plant DNA) extracted.
2. Bacterial genes are inserted into plasmid via genetic modification.
3. Plasmid is returned to the bacterium.
4. Plant is infected with the bacterium.
5. Plant grows a **crown gall**. The cells of the crown gall contain the inserted gene.
6. These cells can be isolated and cultured to grow whole new transgenic plants.

Uses:

- Flood resistance
- Pesticide production
- Herbicide resistance
- Changing the nutrient value of plants:

E.g.

- **Soya beans**
- **Linoleic acid** (polyunsaturated) is replaced by **oleic acid** (monounsaturated), which oxidises less easily and therefore doesn't go off as quickly, and is also healthier.