Decomposition, PCR, gel electrophoresis and forensics

Decomposers and carbon recycling, the polymerase chain reaction, gel electrophoresis, DNA profiling, and estimating the time of death.

Decomposition and the recycling of carbon

Decomposers (bacteria and fungi, also called saprobionts) break down **dead organic matter** by secreting **extracellular enzymes** and absorbing the products. In **respiration** they release **carbon dioxide** back to the atmosphere and recycle other elements, such as **nitrogen** and **phosphorus**, as ions in the soil. Without them, nutrients would remain locked up in dead material and the cycle would stop.

The polymerase chain reaction (PCR)

PCR is used to **amplify** (make millions of copies of) a small sample of DNA in a few hours.

Each cycle has three steps:

1. **Denaturation** at about **95 °C**: the DNA strands separate (hydrogen bonds break).
2. **Annealing** at about **55–65 °C**: short **primers** (single-stranded DNA that is complementary to the ends of the target sequence) bind to the template strands.
3. **Extension** at about **72 °C**: a **heat-stable DNA polymerase** (**Taq polymerase**, from *Thermus aquaticus*) joins free nucleotides to the primers, making a copy of each strand.

The number of copies **doubles** each cycle (2^n copies after n cycles). Ingredients: the DNA, primers, free nucleotides, Taq polymerase, and a buffer.

> **Taq polymerase is used because it is not denatured at 95 °C. A normal polymerase would be denatured at that temperature and would have to be replaced in each cycle.**

Gel electrophoresis

Gel electrophoresis separates **DNA fragments** by **length**.

1. DNA is cut into fragments by **restriction endonucleases**.
2. The fragments are placed in **wells** at one end of an **agarose gel**, which is covered in a **buffer** that conducts electricity.
3. An **electric current** is applied. DNA is **negatively charged** (from the phosphate groups), so it moves towards the **positive electrode** (anode).

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4. **Smaller fragments** move **further** and faster through the gel, as they are less hindered. The fragments are separated in order of size.
5. The DNA is made visible with a **fluorescent dye** under UV or with a radioactive probe.

A **DNA ladder** (fragments of known size) is run alongside to estimate the sizes.

DNA profiling

A **DNA profile** (genetic fingerprint) is a pattern of DNA fragments that is **unique** to an individual (except for identical twins). It is based on **short tandem repeats (STRs)**, non-coding sections of DNA that have **repeated sequences** and vary in the number of repeats between individuals.

Method:

1. **Extract** DNA from a sample (blood, hair root, saliva).
2. **Amplify** specific STRs by **PCR** (with fluorescently labelled primers).
3. **Separate** the fragments by **gel electrophoresis** (or capillary electrophoresis).
4. Compare the **banding patterns**.

Uses:

- **Forensic science**: matching DNA at a crime scene to a suspect.
- **Paternity** and determining **genetic relationships**: a child has bands from both parents.
- Identifying **victims**, and **breeding programmes** (in plants and animals), and diagnosing **genetic diseases**.

Time of death

A forensic scientist estimates the **time since death** (post-mortem interval) of a mammal from:

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Evidence	How it is used
Body temperature	The body cools from 37 °C toward the surrounding temperature (the rate depends on the environment, the size of the body and the clothing). A **cooling curve** gives an estimate
Degree of muscle contraction (rigor mortis)	After death, ATP is no longer made, so the **actin and myosin remain linked** and the muscles stiffen (starting about 2–6 hours after death, full at about 12 hours) and then relax as the muscle proteins break down (after about 24–36 hours)
Extent of decomposition	Autolysis (self-digestion by the body's own enzymes), then bacterial action, changing colour and bloating of the body
Stage of succession	Different **organisms** colonise a body at different stages, in a known sequence (succession)
Forensic entomology	The **life cycle stage** of **insects** such as **blowfly** eggs, larvae (maggots) and pupae on the body. The age of the oldest larvae tells how long ago the eggs were laid, which depends on temperature

NOTE

All estimates are **approximate** and affected by temperature, humidity, burial, clothing and the cause of death. Using several types of evidence gives a more reliable result than any one.

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