

Decay, DNA techniques and forensics

Revision notes · Decomposition and carbon recycling, PCR, gel electrophoresis, DNA profiling, and the evidence used to estimate time of death.

For Pearson Edexcel International AS Biology XBI11 and International A Level Biology YBI11. **A Level only** is IA2 content (Units 4 to 6), not needed for the International AS.

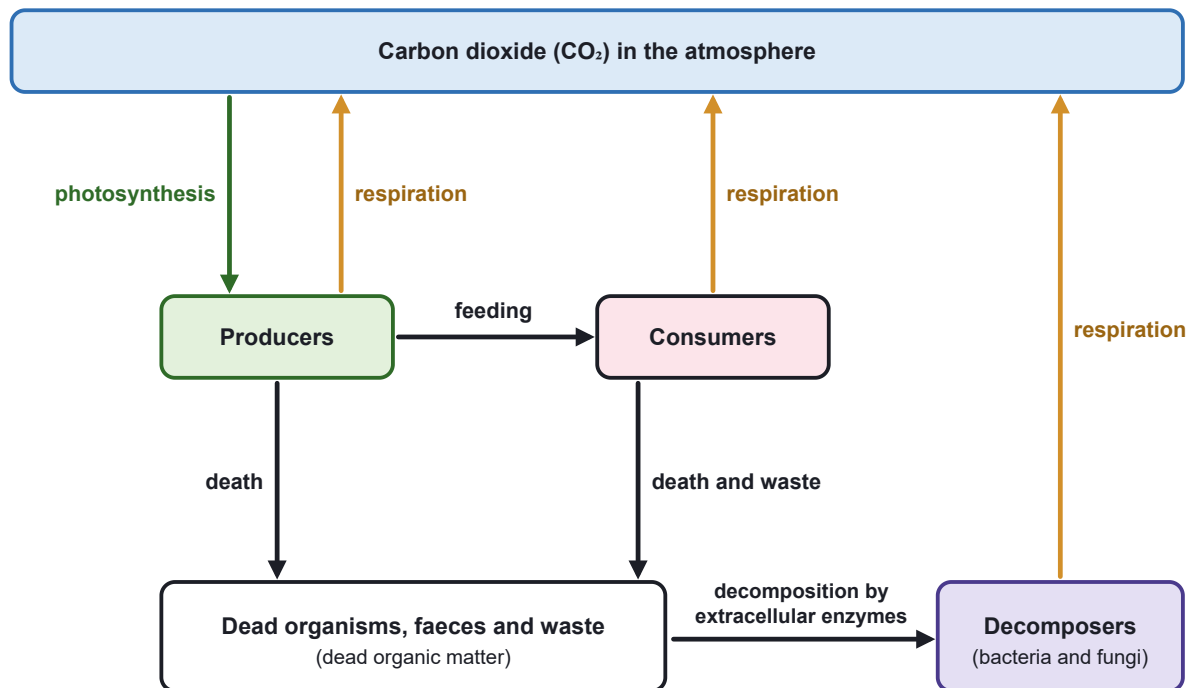
Decay, DNA techniques and forensics **Spec 6.16–6.20** **A Level only**

Microorganisms, decomposition and recycling carbon **Spec 6.16**

Decomposers are mostly saprotrophic bacteria and fungi. They feed by **extracellular digestion**: they secrete enzymes (proteases, lipases, carbohydrases such as cellulase) on to dead plants and animals, faeces and other waste. The enzymes hydrolyse large molecules into soluble ones (amino acids, fatty acids, glucose), which the microorganism absorbs. They use some of these for growth and respiration.

1. Producers take carbon dioxide from the atmosphere and fix it into organic compounds by photosynthesis.
2. Carbon passes to consumers when they feed.
3. Producers, consumers and decomposers all release carbon dioxide into the atmosphere by respiration.
4. Dead organisms, faeces and waste are digested by saprotrophs. Carbon in the organic molecules is converted to carbon dioxide when the decomposers respire, and the carbon is available again to producers.

Decomposition is fastest when conditions suit the microorganisms: warm temperatures (enzymes near their optimum), moisture, oxygen for aerobic respiration, and a suitable pH. Cold, dry, waterlogged, anaerobic or acidic conditions slow it, which is why peat and bog bodies persist. Without microorganisms, carbon would remain locked up in dead matter and producers would run short of carbon dioxide.



The **carbon cycle**, showing the role of decomposers.

Exam tip: Say that decomposers secrete extracellular enzymes, absorb the digested products and respire, releasing CO₂. 'They break down dead things' alone gains no marks.

Amplifying DNA by the polymerase chain reaction Spec 6.17

The **polymerase chain reaction (PCR)** makes millions of copies of a particular piece of DNA from a very small sample, such as a hair root at a crime scene. The tube contains:

- the DNA template (the sample)
- two **primers**: short single-stranded DNA sequences complementary to the start of each strand of the target sequence
- free DNA nucleotides (dATP, dTTP, dCTP, dGTP)
- **Taq polymerase**, a DNA polymerase from the bacterium *Thermus aquaticus* that is heat-stable and so is not denatured at 95 °C
- a buffer solution with magnesium ions

1. Denaturation: heat to about 95 °C. Hydrogen bonds between the complementary base pairs break, separating the two strands.
2. Annealing: cool to about 55 to 65 °C. Primers bind (anneal) by hydrogen bonds to their complementary sequences at the ends of the target DNA on each strand.
3. Extension: heat to about 72 °C, the optimum for Taq polymerase. It adds free nucleotides to the 3' end of each primer, building a complementary strand, to make two double-stranded copies.
4. The cycle is repeated, typically 25 to 35 times in an automated thermal cycler. The amount of DNA doubles each cycle, so after n cycles there are 2^n copies of the target from each original molecule.

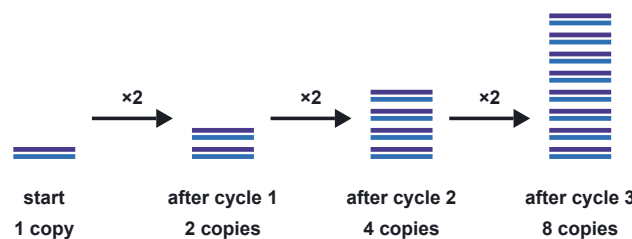
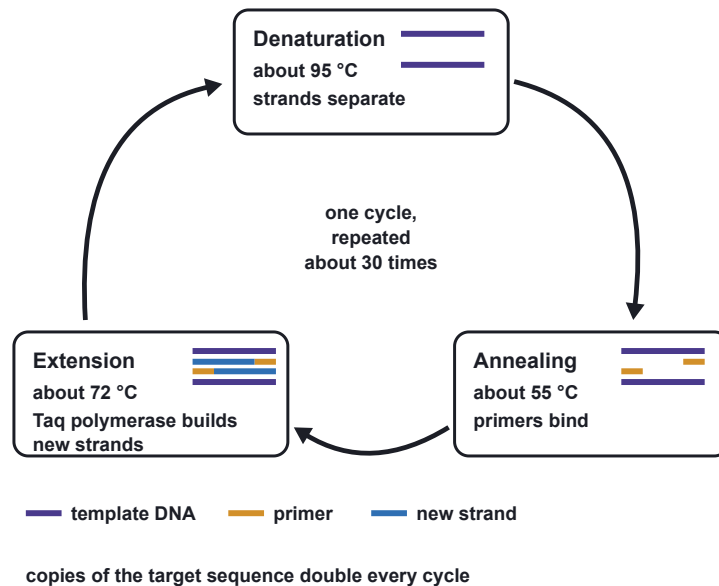
The primers make the technique specific for one sequence, and the use of a heat-stable enzyme means that the polymerase does not have to be added again each cycle. A very small sample can be amplified enough to be analysed. Contamination with other DNA is a risk, so great care is needed.

Amplification

How many copies of a DNA sequence would there be after 25 cycles of PCR starting from one molecule?

1. The number doubles each cycle, so copies = 2^n .
2. $2^{25} = 33\,554\,432$.

Answer: About 3.4×10^7 copies



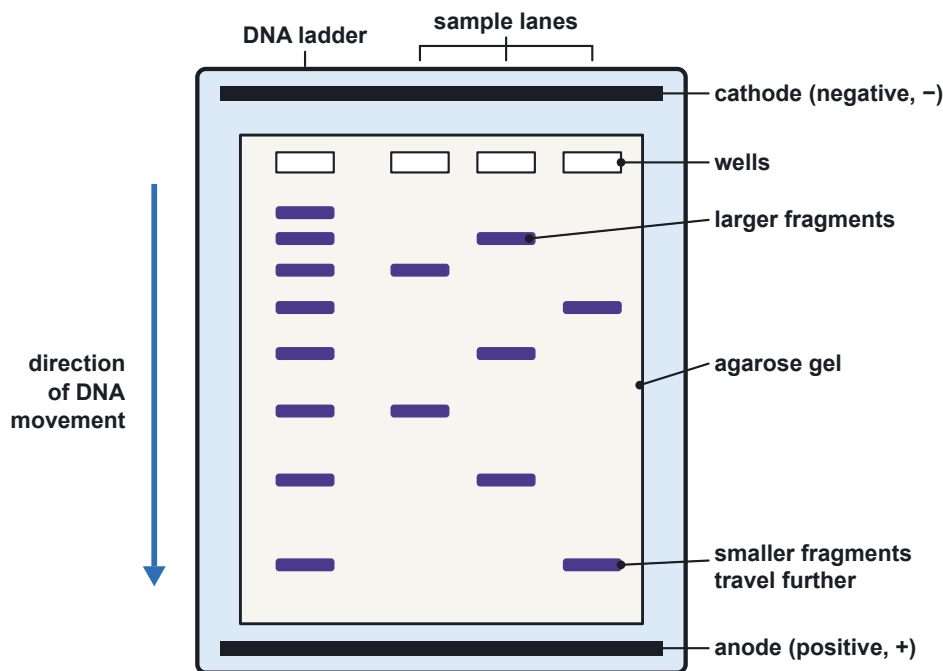
One cycle of **PCR**: the number of copies doubles every cycle.

Separating DNA fragments by gel electrophoresis Spec 6.18

1. DNA is cut into fragments with restriction enzymes (or amplified by PCR).
2. A gel made of agarose (a porous polysaccharide) is covered with a buffer solution that conducts electricity. Samples, mixed with loading dye to make them dense and visible, are put into wells at the negative end. A mixture of fragments of known lengths (a **DNA ladder**) is put in one well.
3. An electric current is applied. DNA is negatively charged because of the phosphate groups, so it moves through the gel towards the positive electrode (anode).
4. The gel acts as a mesh. Shorter fragments move through it more easily, so they travel further in a given time, and longer fragments move more slowly. Fragments of the same length form a band.
5. The DNA is made visible by staining with a fluorescent dye that binds to DNA and is viewed in UV light, or by DNA probes that carry a fluorescent or radioactive label.

The length of a fragment is estimated by comparing how far its band has moved with the bands of the DNA ladder. A graph of $\log_{10}$ fragment length against distance moved is roughly a straight line.

Exam tip: The two key ideas are charge (negative phosphate groups, so moves to the anode) and size (smaller fragments move further through the gel). Fragments are separated by length, not by mass of the whole sample.



Gel electrophoresis: DNA is negatively charged, so it moves towards the **anode**; smaller fragments move further.

DNA profiling Spec 6.19

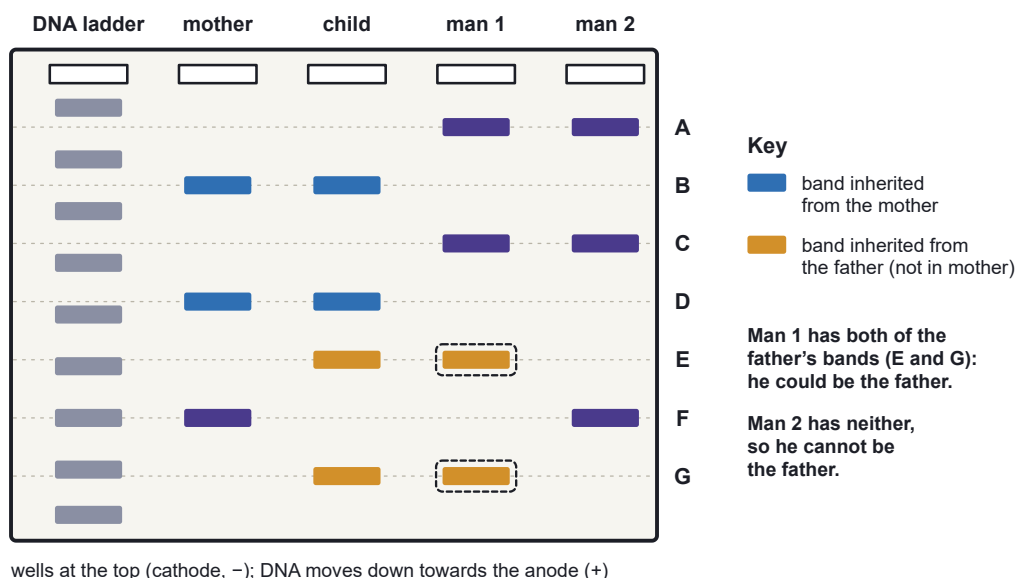
Much of the genome is non-coding DNA, which contains short sequences repeated many times in a row (short tandem repeats, STRs). The number of repeats at each position (locus) differs between individuals, so the length of the fragment differs. Each person inherits one set from each parent, so, apart from identical twins, no two people have the same pattern across several loci.

1. DNA is extracted from a sample (blood, hair root, saliva, tissue or seed).
2. Regions containing STRs are amplified by PCR using primers (often labelled with a fluorescent tag).
3. The fragments are separated by gel electrophoresis and detected, giving a pattern of bands (the DNA profile).
4. Profiles are compared. Bands at the same position in two samples match.

Uses

Uses of DNA profiling	
Use	How profiles are used
Identification: forensic	DNA from a crime scene is compared with DNA from suspects or a database; a match of many bands makes it very unlikely that the DNA is from anyone else
Identification: victims, species and products	Identifies remains; identifies species or individual animals, for example illegal ivory or poached animals; identifies plant varieties or checks the origin of food or timber
Genetic relationships: individuals	Paternity and maternity: every band in a child's profile must be present in the mother or the father. The father's bands that the child has but the mother does not show who the father could be
Genetic relationships: plants and animals	Measures the relatedness of individuals in a breeding programme or wild population, so that inbreeding can be reduced; used to show relationships between species and varieties

Maths skill: Probability: if the chance of a match at one locus is 1 in 10 and loci are independent, the chance of a match at five loci is $(1/10)^5 = 1$ in 100 000. More loci mean a more reliable identification.



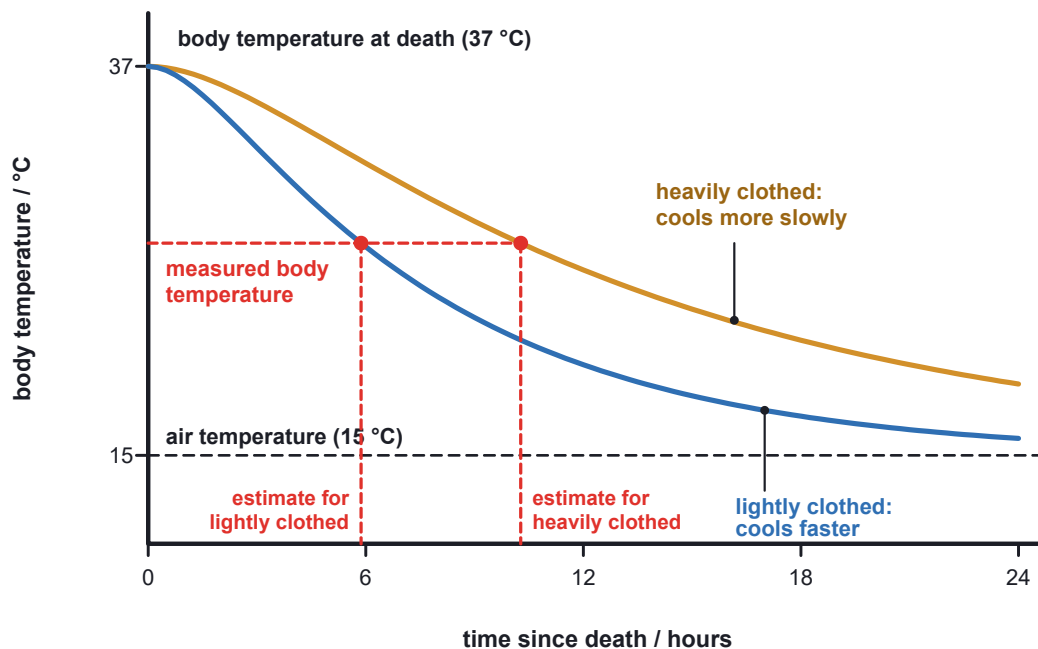
A **paternity** test: every band in the child must come from the mother or the father, so only man 1 could be the father.

Estimating time of death Spec 6.20

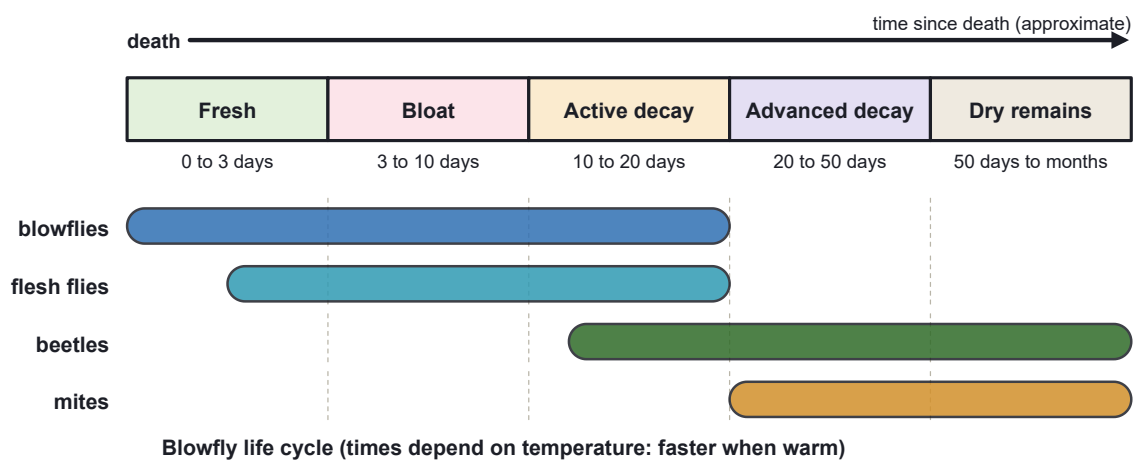
Evidence used to estimate time of death	
Evidence	What is looked at and why it changes with time
Body temperature	After death, metabolism stops and the body cools towards the temperature of its surroundings. Core (rectal) temperature is measured and compared with 37 °C (cooling curve). Speed of cooling depends on air temperature, clothing, body mass and wind or water. Most useful in the first 24 hours or so
Degree of muscle contraction (rigor mortis)	No new ATP is made after death, so myosin heads cannot detach from actin and muscles stay contracted and stiff. It starts in smaller muscles in the face a few hours after death, spreads, and passes off after about 1 to 2 days as muscle proteins are broken down by enzymes. Warm conditions make it start and end sooner
Extent of decomposition	Bacteria in the gut, and enzymes from dead cells (autolysis), digest tissues. In sequence the body shows discolouration, bloating due to gases, softening and liquefaction of tissues and finally dry remains and bones. Slower in cold, dry or burial conditions
Stage of succession	Organisms colonise a body in a sequence as its condition changes: blowflies arrive within minutes to hours, then flesh flies, then beetles that feed on drier tissue, later mites and species that feed on hair and bone. The community present shows the stage of decay
Forensic entomology	Blowflies lay eggs on a body, which hatch into larvae (maggots), grow through stages (instars), pupate and emerge as adults. Species and size of the oldest larvae, or the stage of pupae, give the time since the eggs were laid, allowing for temperature (growth is faster when warm). Eggs are laid only after death, so the age of the oldest larvae gives a minimum time since death

Each method is only an estimate, and the earlier the body is found, the smaller the error. Conditions such as air temperature, burial, water, clothing and whether the body is indoors alter all of the changes. A forensic scientist therefore uses evidence from more than one method, records the environmental conditions at the scene and takes samples of insects at once.

Exam tip: Always say what changes and why: 'rigor mortis occurs because ATP is no longer produced, so myosin heads cannot detach from actin'. For succession, say that different species arrive as the condition of the body changes.



Body cooling curves: a measured temperature gives an estimate of time since death, which depends on clothing.



Times are approximate: decomposition is faster in warm, moist conditions and slower in cold, dry ones.

Decomposition and succession: different insects colonise a body as its condition changes (times approximate).

Quick check

- 1 . How do saprotrophs digest dead matter?
- 2 . Why is Taq polymerase used in PCR?
- 3 . Why do shorter DNA fragments travel further in gel electrophoresis?
- 4 . What is an STR?
- 5 . Why does rigor mortis happen?

Quick check answers

Decay, DNA techniques and forensics

1. They secrete extracellular enzymes on to it and absorb the soluble products.
2. It is heat-stable, so it is not denatured at about 95 °C and works at 72 °C.
3. They move more easily through the mesh of the gel.
4. A short tandem repeat: a short sequence of non-coding DNA repeated many times, with a different number of repeats in different people.
5. ATP is no longer made, so myosin heads cannot detach from actin and muscles stay contracted.