DP IB Biology: SL

Your notes

3.4 Genetic Modification & Biotechnology

Contents

3.4.1 Electrophoresis & PCR

Your notes

Gel Electrophoresis

Gel electrophoresis is used to separate proteins or fragments of DNA according to size

- **Gel electrophoresis** is a technique used widely in the analysis of DNA, RNA, and proteins
- During electrophoresis, the **molecules** are **separated with an electric current** according to their **size or mass** and their **net (overall) charge**
- This separation occurs because of:
  - The **electrical charge** molecules carry:
    - Positively charged molecules will move towards the cathode (negative pole), whereas negatively charged molecules will move towards the anode (positive pole) e.g. **DNA is negatively charged** due to the **phosphate** groups and thus, when placed in an electric current, the molecules **move towards the anode**
  - The **different sizes** of the molecules:
    - Different sized molecules move through the gel (agarose for DNA and polyacrylamide for proteins) at different rates. The tiny pores in the gel result in **smaller molecules** moving **quickly**, whereas **larger molecules** move **slowly**
  - The **type of gel**:
    - Different gels have different sized pores that affect the speed at which the molecules can move through the gel

DNA separation

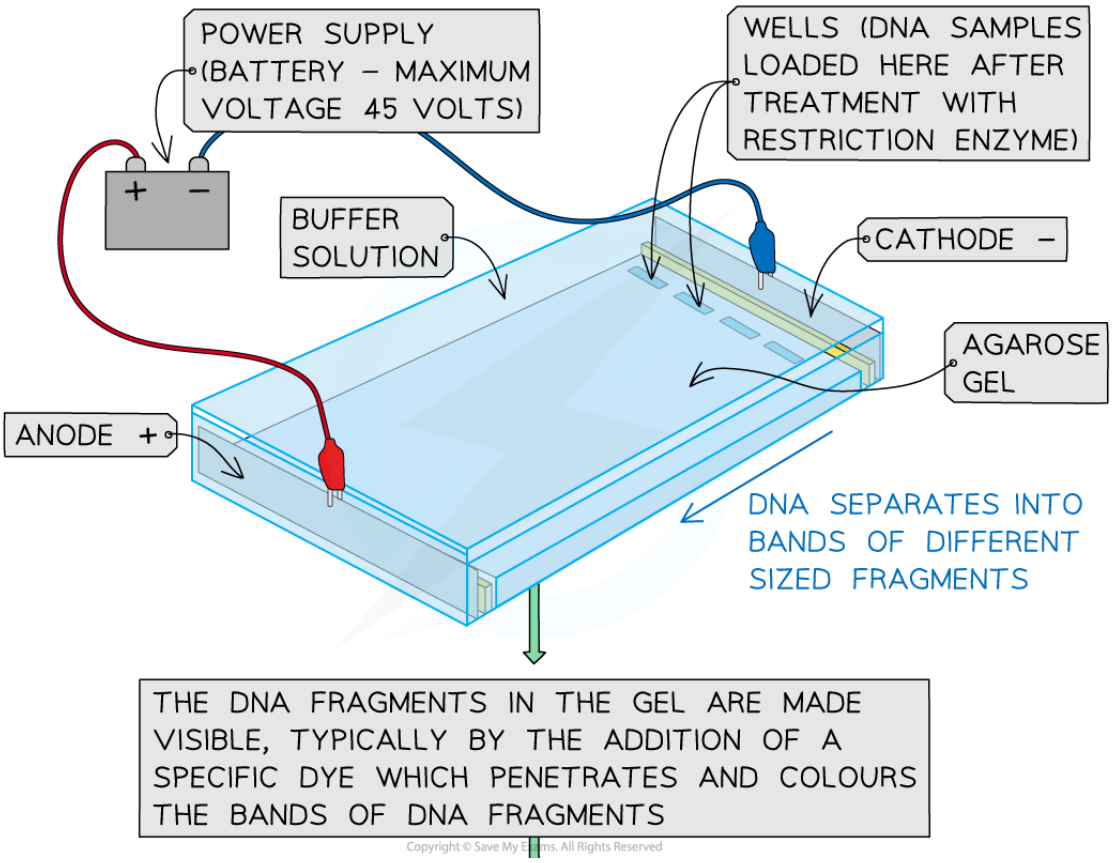
- DNA can be collected from almost anywhere on the body, e.g. the root of a hair or saliva from a cup. After collection, DNA must be prepared for gel electrophoresis so that the **DNA** can be **sequenced** or analysed for **genetic profiling (fingerprinting)**
- To prepare the fragments, scientists must first increase (amplify) the number of DNA molecules by the **Polymerase Chain Reaction** (PCR)
- Then restriction (DNA-cutting) enzymes are used to chop the DNA into fragments

Method

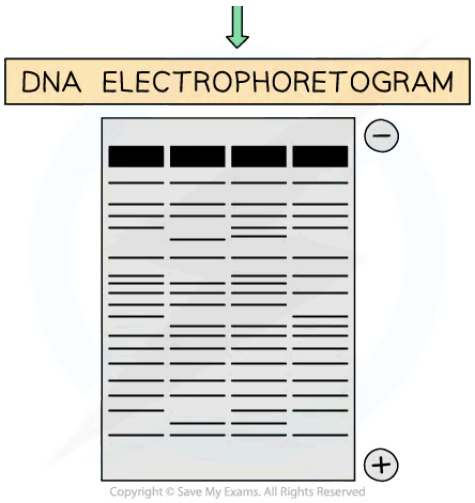
- **To separate the DNA fragments** in gel electrophoresis:

1. Create an **agarose gel** plate in a tank. **Wells** (a series of small rectangular holes) are cut into the gel at one end
2. Submerge the gel in an **electrolyte** solution (a salt solution that conducts electricity) in the tank
3. Load (insert) the DNA fragments into the wells using a **micropipette**
4. Apply an **electrical current** to the tank. The negative electrode must be connected to the end of the plate with the wells as the DNA fragments will then move towards the anode (positive pole) due to the attraction between the negatively charged phosphates of DNA and the anode

5. DNA fragments with a smaller mass (i.e. shorter DNA fragments) will move faster and further from the wells than the larger fragments
6. **The fragments are not visible** so must be transferred onto absorbent paper or nitrocellulose which is then heated to separate the two DNA strands
7. **Probes** are then added to develop a visual output, either:
   - A **radioactive label** (e.g. a phosphorus isotope), which causes the probes to emit radiation that makes the X-ray film go dark, creating a pattern of dark bands
   - A **fluorescent stain or dye** (e.g. ethidium bromide), which fluoresces (shines) when exposed to ultraviolet (UV) light, creating a pattern of coloured bands



Your notes



The process of electrophoresis

Examiner Tip

Remember gel electrophoresis is the separation of molecules according to their size and charge (negatively charged DNA molecules move to the positive pole). Examiners like to ask questions about gel electrophoresis, so make sure you understand each of the different steps in the process.

Your notes

Polymerase Chain Reaction (PCR)


Your notes

PCR can be used to amplify small amounts of DNA

- **Polymerase Chain Reaction** (PCR) is a common molecular biology technique used in most applications of gene technology
  - For example, it is used in DNA profiling (e.g. identification of criminals and determining paternity) or genetic engineering
  - In the **COVID-19 pandemic**, PCR has been used in **routine diagnostic testing** to amplify small amounts of viral RNA
- It can be described as the ***in vitro* method of DNA amplification**
- It is used to produce **large quantities** of specific fragments of DNA or RNA from very small quantities (even just one molecule of DNA or RNA)
  - Using PCR, scientists can produce **billions of identical copies** of the DNA or RNA samples within a few hours, these can then be used for **analysis**

The requirements of PCR

- Each PCR reaction requires:
  - The **target DNA** or **RNA** that is being amplified
  - It's important that **the whole genome is not required** to be copied - only specific sections that vary from one individual to another
    - These sections are identified by adding a **primer sequence** that binds to them
  - **DNA polymerase** - the enzyme used to build the new DNA or RNA strand. The most commonly used polymerase is ***Taq* polymerase** as it comes from a thermophilic bacterium *Thermus aquaticus*
    - This means it **does not denature** at the **high temperature** involved during the first stage of the PCR reaction
  - **Free nucleotides** - used in the construction of the DNA or RNA strands
  - **Buffer solution** - to provide the optimum pH for the reactions to occur in

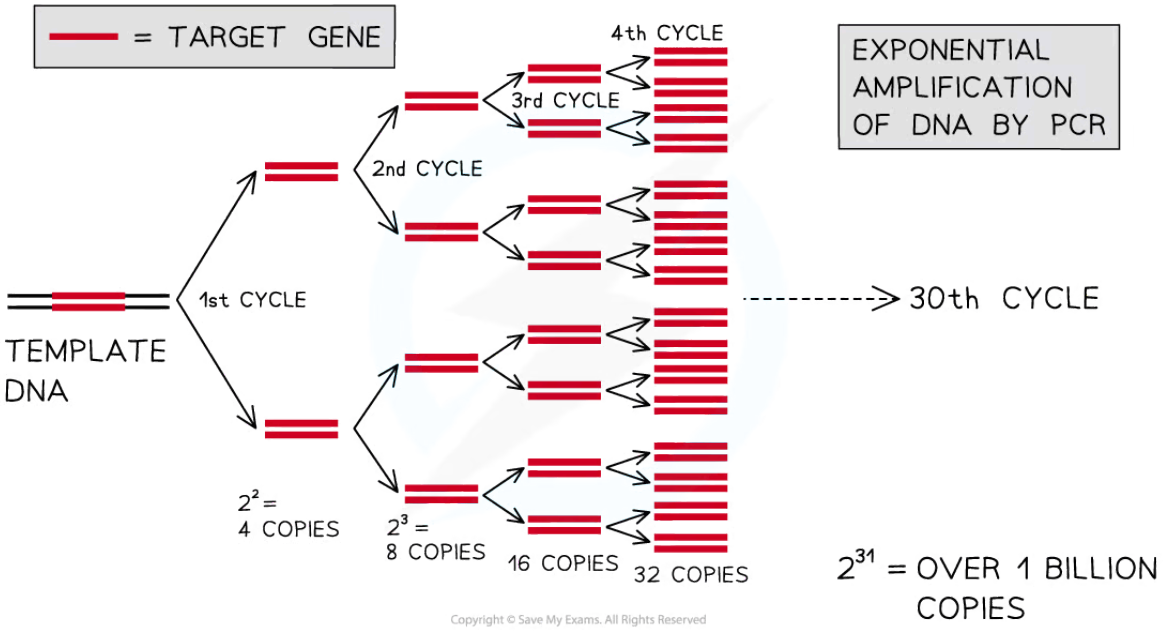
The key stages of PCR

- The PCR process involves **three key stages** per cycle
- In each cycle the DNA is doubled (so in a standard run of 20 cycles a million DNA molecules are produced)
- The PCR process occurs in a piece of specialist equipment called a **thermal cycler,** which automatically provides the **optimal temperature** for each stage and controls the **length of time** spent at each stage
- The three stages are:

1. **Denaturation** – the double-stranded DNA is heated to 95°C which breaks the hydrogen bonds that bond the two DNA strands together
2. **Annealing** – the temperature is decreased to between 50 - 60°C so that primers can anneal to the ends of the single strands of DNA

3. **Elongation / Extension** – the temperature is increased to 72°C for at least a minute, as this is the optimum temperature for *Taq* polymerase to build the complementary strands of DNA to produce the new identical double-stranded DNA molecules

- **Each whole cycle takes a few minutes**, so 30 cycles can take just a few hours and can generate 2^{31} (over 1 billion) copies of a gene from a single DNA molecule, by **exponential amplification**



Target DNA sequences can be copied exponentially by PCR to generate billions of copies in a short time

Examiner Tip

You don't need to know the detail of the three stages and the temperatures the reactions occur at during the different stages. However, you must know why the *Taq* polymerase is used in PCR (from Topic 2.6.3). The main learning point is that PCR can be used to amplify very small amounts of DNA into large numbers of molecules for analysis.

Your notes

3.4.2 DNA Profiling


Your notes

Use of DNA Profiling

DNA profiling involves comparison of DNA

- **DNA profiling** (genetic fingerprinting) enables scientists to **identify suspects for a crime** and **identify corpses** because every person (apart from identical twins) has repeating, short, non-coding regions of DNA (20 to 50 bases long) that are unique to them
- To **create a DNA profile** from the DNA being tested scientists complete the following in sequence:

1. **Obtain the DNA**, which can be extracted from the root of a hair, a spot of blood, semen or saliva
2. Increase the quantity of DNA by using **PCR** to produce **large quantities** of the required fragment of DNA from very small samples (even just one molecule of DNA or RNA).
3. Use **restriction endonucleases** to cut the amplified DNA molecules into fragments
4. Separate the fragments using **gel electrophoresis**
5. Add **radioactive or fluorescent probes** that are complementary and therefore bind to specific DNA sequences
6. X-ray images are produced or UV light is used to produce images of the fluorescent labels glowing
7. These images contain **patterns of bars** (the DNA profile) which are then **analysed and compared**

DNA Profiling

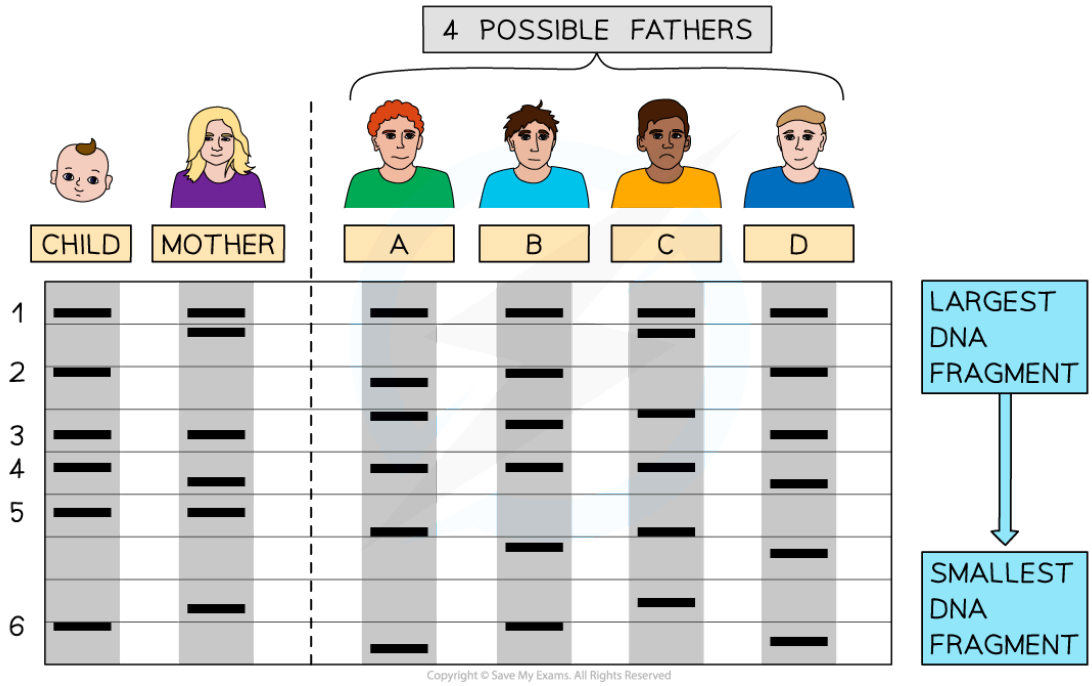
Your notes

Use of DNA profiling in Paternity Investigations

- A man may sometimes **deny being the father** of a child to evade parenting responsibilities
- A woman may not know for sure which of her recent sexual partners is the father of a child
- A child may wish to know definitively who his/her father is to **be aware of possible inherited illnesses** that might affect him/her in future
- DNA profiles of the mother and child are compared, along with the profile of the alleged father (**all three** are needed)
- **Patterns of bands are compared** on all three genetic profiles
  - Any band that appears in the child's profile **must show in either the mother's or father's profiles**; if not, the alleged true father is a different man

Worked example

Who's the Father? – Use the DNA profiles of all 6 people shown to work out who the child's father is



Remember, any band showing in the child's profile must be present in the mother **OR** father's profile, **OR** both. If not, that man is not the child's father.

Step 1: Look at the child's first DNA band (labelled 1)

The mother possesses this same band, so the child could have inherited that DNA from its mother. It is therefore needless to look at whether any of the men possess that band

Step 2: Look at the child's second DNA band (labelled 2)

The mother does not possess this band, so the child must have inherited it from its father. Only men B and D possess this band, so men A and C are eliminated

Step 3: Look at the child's third DNA band (labelled 3)

As with band 1, the mother possesses this same band, so the child could have inherited that DNA from its mother. It is therefore needless to look at whether any of the men possess that band

Step 4: Look at the child's fourth DNA band (labelled 4)

The mother does not possess this band, so the child must have inherited it from its father. Only men A, B and C possess this band, but A and C have already been eliminated

Step 5: Conclude that B is the father

Step 6: Look for supporting evidence from band 6

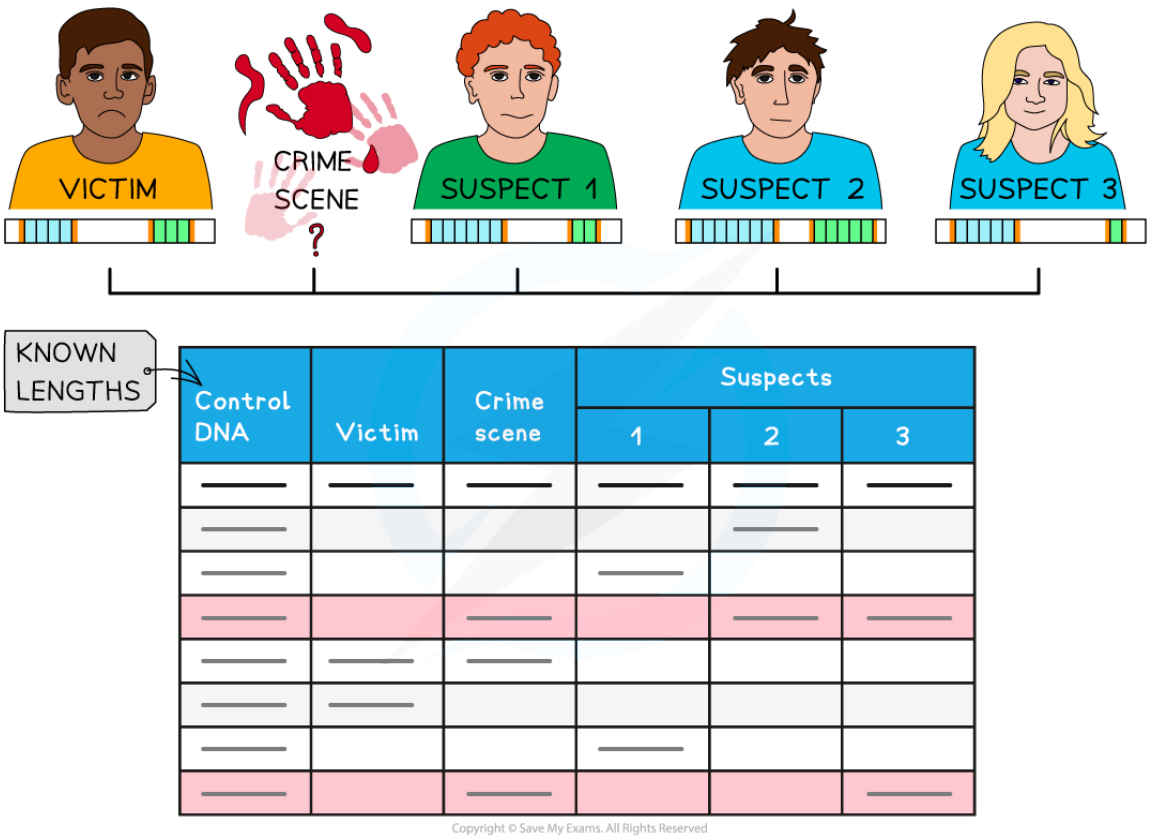
The mother does not possess this band, and the only man who possesses it is B. **This reinforces the conclusion that Man B is the child's father**


Your notes

Use of DNA profiling in Forensic Investigations

- DNA profiling has been used by forensic scientists to **identify suspects** of crimes
  - Samples of body cells or fluids (eg. blood, saliva, hair, semen) are taken from the crime scene or victims body (eg. rape victims)
  - DNA is **removed** and **profiled**
  - The profile is **compared to samples from the suspect** (or criminal DNA database), victim and people with no connection to the crime (control samples)
  - Care must be taken to **avoid contamination** of the samples
- DNA profiling can also be used in forensics to **identify bodies** or body parts that are unidentifiable (eg. too badly decomposed or parts remaining after a severe fire)
- DNA profiling from a crime scene can also **eliminate innocent people** whose DNA may happen to appear there

Your notes



Using DNA profiling in criminal investigations. Suspect 3 has the most fragments in common with the crime scene DNA so it is likely that Suspect 3 is the culprit.

Examiner Tip

In the exam, you will be expected to interpret the results of gel electrophoresis experiments used to separate DNA fragments. For example, you will be given a few different genetic fingerprints and will have to match the victim to the crime or determine the parents of children. In these questions, you need to look for the most bands in common or a combination of parents' fingerprints that covers all the child's bands.

3.4.3 Genetic Modification

Your notes

Genetic Modification

Genetic modification is carried out by gene transfer between species

- **Genetic modification** is a term usually used to refer to the transfer of DNA sequences from one species to another
- The key feature of the genetic code that makes this possible is that it is **universal**, meaning that almost every organism uses the same four nitrogenous bases – A, T, C & G. There are a few exceptions
  - Additionally the **same codons code for the same amino acids in all living things** (meaning that genetic information is transferable between species)
- Thus scientists have been able to change an organism's DNA artificially by combining lengths of nucleotides from **different sources** (typically the nucleotides are from different species)
- If an organism contains nucleotide sequences from a different species it is called a **transgenic** organism
- DNA that has been introduced into the genome of another organism is called **recombinant DNA** (rDNA)
- Any organism that has introduced genetic material is a **genetically modified organism** (GMO)
- The mechanisms of **transcription** and **translation** are also **universal** which means that the transferred DNA can be translated within cells of the genetically modified organism

Recombinant DNA technology

- This form of genetic modification involves **the transfer of fragments of DNA** from **one organism/species into another** organism/species
- The resulting genetically modified organism will then contain **recombinant DNA** and will be a **Genetically Modified Organism** (GMO)
- Example
  - A gene from the bacterium *Bacillus thuringiensis* (*Bt* for short) codes for a **toxin** that has **insecticide** properties
  - This gene has useful properties in commercial maize plants (*Zea mays*), so has been transferred into **transgenic maize plants** to make them less susceptible to insect pests, **improving agricultural productivity** as a result

Your notes

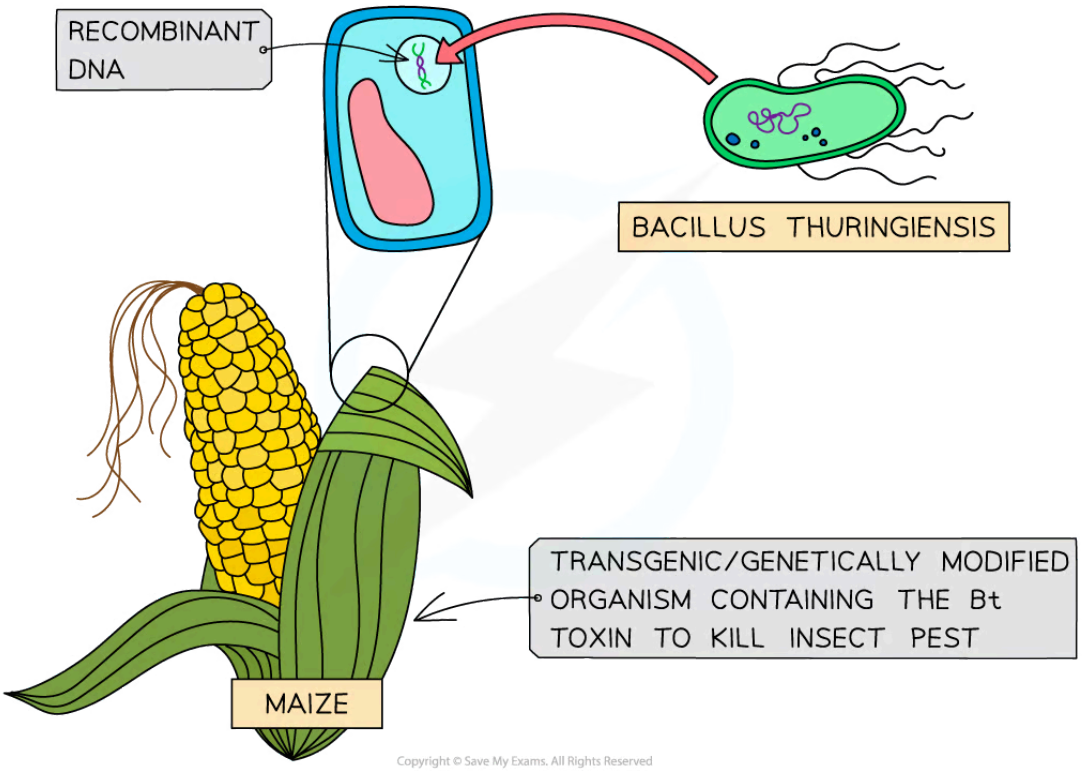


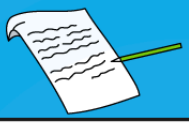
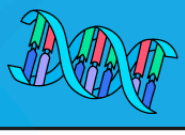
***Illustration of a maize plant that has recombinant DNA (DNA from* Bacillus thuringiensis)**

Uses of genetic modification

- Because all genes code for proteins, **useful proteins** can be manufactured by the creating of transgenic organisms
- Some of the key uses of genetic modification include the genetic modification of:
  - **Crops** to increase crop yield through resistance to drought, disease, pesticides and herbicides; or to provide increased nutritional value (e.g. golden rice)
  - **Livestock** to give disease and pest resistance, increased productivity and new characteristics (eg. goats that produce milk containing spider silk)
  - **Bacteria** to produce medicines e.g. insulin. Additionally bacterial can be modified to decompose toxic pollutants or carry out large scale chemical production

Analogy: Essay Writing and Recombinant DNA

- Creating **transgenic organisms** is rather like **copying and pasting** some text from one of your previous essays into the one that you are currently writing
- If you believe that the essay that you are currently writing can be strengthened by the use of some text from another essay that you have previously written, it is a common practice to use the computer's **copy and paste** function to transfer text in one block without having to retype it
- This has similar features to genetic modification in the creation of a transgenic organism

Stage	Essay writing 	Genetic modification 
Identification	identify useful text from first essay	identify useful gene in one organism
Same language?	yes – english to english	yes – universal genetic code (a, c, g, t)
Identify start point	place mouse cursor at beginning of text to be copied	find restriction site at the beginning of the gene to be copied
Select material to be copied	select down to the end of the text to be copied	find restriction site at the end of the gene to be copied
Cut	right–click–cut or ctrl–x	restriction endonuclease enzymes
Vector	computer clipboard (memory)	eg. a plasmid
Select destination	place mouse cursor at insertion point	restriction endonuclease enzyme to open up dna at the insertion point
Paste	right–click–paste or ctrl–v	ligase enzyme to merge recombinant plasmid into destination genome
Function check	does the pasted text convey the point i wish to make in this essay, in the right place?	is the recombinant gene being expressed effectively in the transgenic organism? are there no detrimental side effects?
Ethical considerations	have i pasted my own text and not plagiarised somebody else's work?	many ethical considerations (see later revision notes)

Your notes

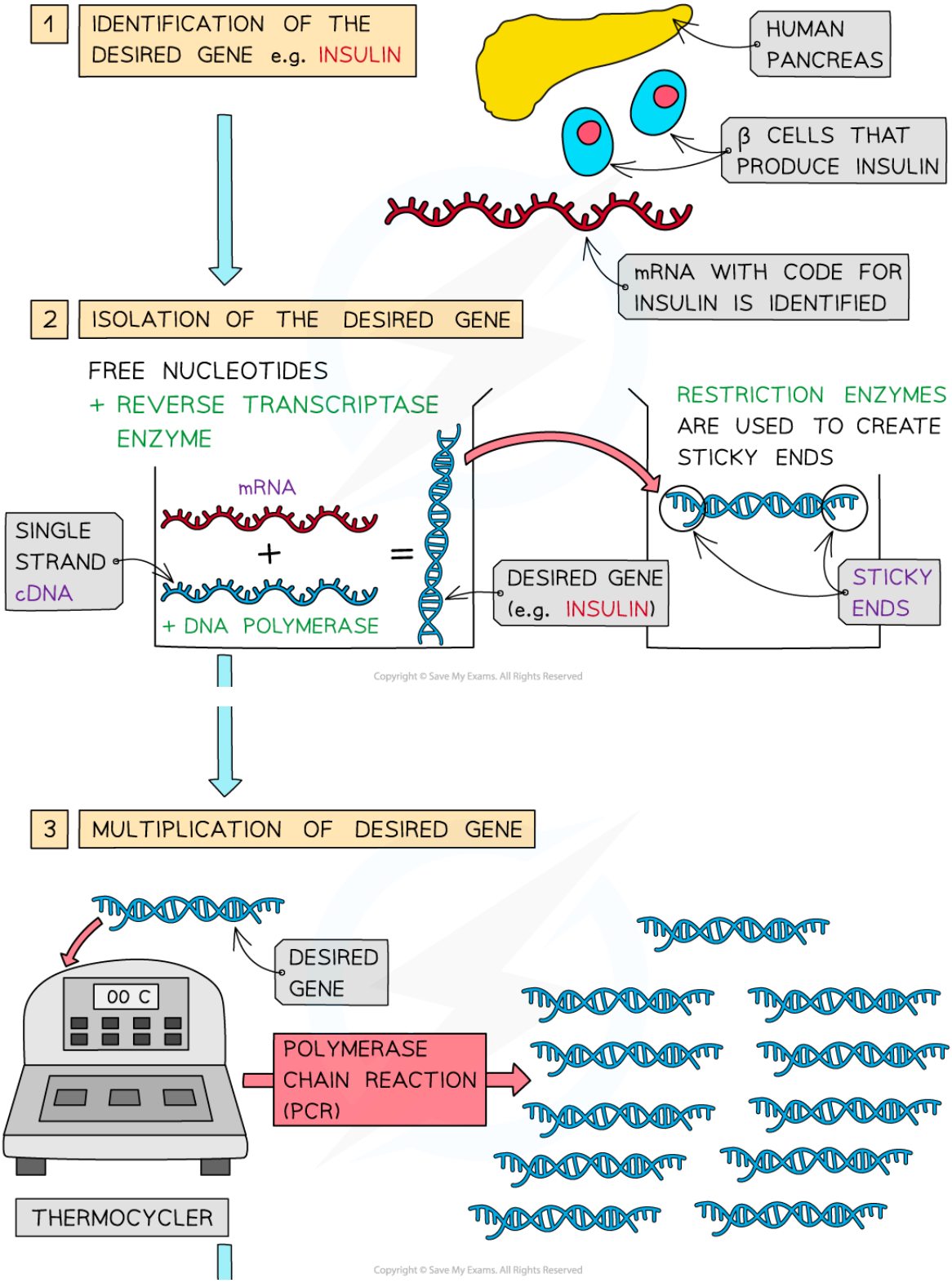
Genetic Modification: Enzymes


Your notes

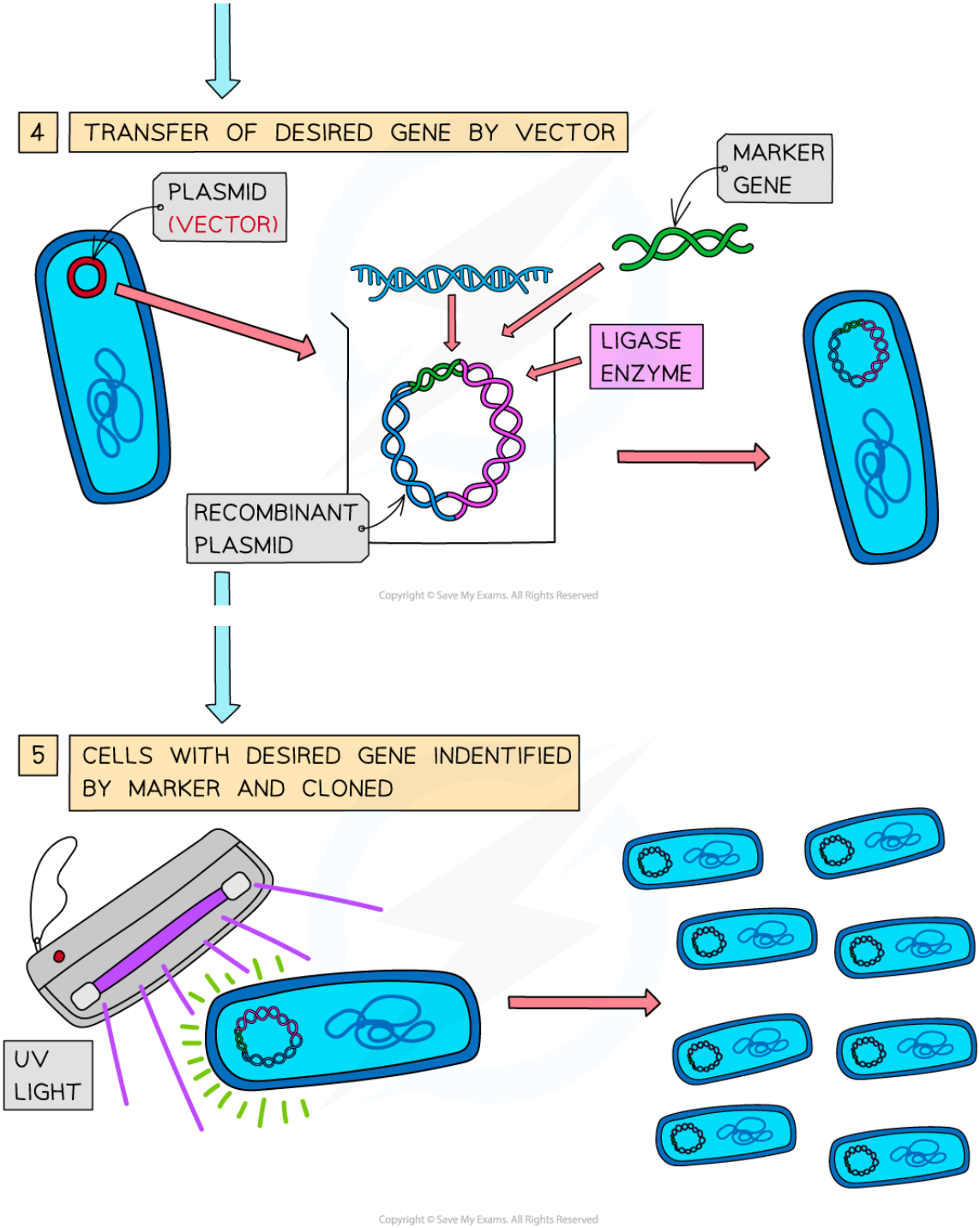
Gene transfer to bacteria using plasmids makes use of restriction endonucleases and DNA ligase

- In order for an organism to be genetically modified the following steps must be taken:
  - **Identification** of the **DNA fragment** or **gene**
  - **Isolation** of the desired DNA fragment (either using restriction endonucleases or reverse transcriptase)
  - **Multiplication** of the DNA fragment (using polymerase chain reaction - PCR)
  - **Transfer** into the organism using a **vector** (e.g. plasmids, viruses, liposomes)
    - A plasmid is a **small, circular loop of DNA** found in the cytoplasm of bacteria, **separate from its main loop** of DNA
    - Plasmids form part of the **bacterial genome**
    - Plasmids are extremely useful in genetic modification because of their **small size** and their **ability to be manipulated separately** to the bacterium's main genome
  - **Identification** of the cells with the new DNA fragment (by using a **marker**), which is then cloned
- Geneticists need the following 'tools' to modify an organism:
  - **Enzymes**
    - **Restriction endonucleases** - used to cut genes at specific base sequences (restriction sites). Different restriction enzymes cut at different restriction sites
    - These can create **sticky ends**
    - **Ligase** - used to join together the cut ends of DNA by forming covalent bonds and sealing up nicks where fragments have not quite been joined firmly with covalent bonds
    - **Reverse transcriptase** - used to build double-stranded DNA from single-stranded RNA
      - This DNA is called **cDNA** (complementary DNA)
  - **Vectors** - used to deliver DNA fragments into a cell
    - **Plasmids** - transfer DNA into bacteria or yeast
    - **Viruses** - transfer DNA into human cells or bacteria
    - **Liposomes** - fuse with cell membranes to transfer DNA into cells
  - **Markers** - genes that code for identifiable substances that can be tracked
    - eg. **Fluorescent**
    - such as green fluorescent protein (GFP) which fluoresces under UV light

Your notes



Your notes



An overview of the steps taken to genetically modify an organism (in this case bacteria are being genetically modified to produce human insulin)

NOS: Assessing risks associated with scientific research; scientists attempt to assess the risks associated with genetically modified crops or livestock


Your notes

- There are **obvious benefits** of genetically modified organisms being able to express useful genes for human gain
- Nevertheless, there are some **potential risks** that this technology may raise, which scientists (and society in general) need to evaluate alongside the benefits
  - For example, there was much concern that using microorganisms in genetic modification could spread pathogenic disease more widely than had been the case before
- This has led to **intense debates** between scientists and **within wider society** about the role that genetically modified crops can play in the world
- This topic generates **a lot of publicity**, some parts of it better-informed scientifically than others
- Scientists must ask:
  - What are the **risks of an accident** or other harmful effects of using GMOs in agriculture?
  - How **dangerous** could those effects be?
- Many scientific innovations, like GMO crops, appear at first glance to be **a great leap forward** to improve the fortunes of humans as a species
- However, the science can be used in ways that are **morally questionable** (such as rapid generation of profits)
- This can lead to **unexpected problems**, as set out in the possible risks section above
- It is important for humans from all walks of life, informed by scientists, to:
  - Conduct **ethical discussions**
  - Carry out **risk-benefit analysis** and **risk assessment**
  - Apply the **precautionary principle**
    - When a discovery raises a significant threat of harm to the environment or human health, there should be **an assumption that harm will be caused**, until evidence is put forward to the contrary
- Like all of science, claims and hypotheses have to backed up with **experimental evidence**
  - Experiments have to be **controlled, reliable** and **repeatable** in order to draw meaningful conclusions
  - One such example is the effect of *Bacillus thuringiensis (Bt)* toxin-containing pollen in maize plants, on the distribution and health of monarch butterfly larvae

Examiner Tip

When answering questions about genetic modification you should remember to include the names of any enzymes (**restriction endonucleases**, **reverse transcriptase**, **ligase**) involved **and** mention that **vectors** (transfer the desired gene) are also used.

Genetic Modification of Crops: Risks & benefits


Your notes

NOS: Assessing risks associated with scientific research – scientists attempt to assess the risks associated with genetically modified crops or livestock

- Although plants and animals have been genetically modified to produce proteins used in medicine, the main purpose for genetically modifying them is to meet the **global demand for food**
- The benefits of using genetic modification rather than the more traditional selective breeding techniques to solve the global demand for food are:
  - Organisms with the **desired characteristics** are **produced more quickly**
  - **All organisms** will contain the desired characteristic (there is no chance that recessive allele may arise in the population)
  - The desired characteristic may **come from a different species/kingdom**
- Companies that produce genetically modified (GM) seed are **very skilled** at explaining the benefits of their use
- The companies make claims about **improved crop yields** and reduction in the use of chemical pesticides/herbicides
- These **claims make good sense** at first, in a world where a **rapidly growing human population** needs a reliable supply of food

Potential benefits of GM crops

- **Pest-resistant** crop varieties can be created using genes that produce a toxin
  - This reduces insecticide use on the crop
  - In turn, there is less effect on non-pest insects such as bees in the vicinity of the crop
- **Less ploughing and spraying** of the crop is required, so less machinery (and fuel to run it) is required
- **Crop shelf-life** can be improved, so there is less wastage in the supply chain
  - This makes the land used to grow those crops **more productive**
- Crops can be made **frost-resistant** or **drought-resistant**, allowing farmers on relatively poor agricultural land to grow crops and earn a living
- Crops can be **enriched** eg. with vitamins, to increase their nutritional value
- Herbicide-resistant crops can be created, so that use of herbicides **eliminates competition from other plants**
  - More of the crop can grow as it is not competing with other plants for sunlight, space, soil nutrients etc.
- **Disease-resistant varieties** can grow which again, **increases crop yields**

Potential risks of GM crops

- Many people object to the use of GMOs in **food production** due to a **lack of long-term research** on the effects on human health
  - It is unknown whether it will cause allergies or be toxic over time (although there has been no evidence to suggest this would occur to date)

- Organic farmers have claimed that the pollen from GM crops may **contaminate nearby non-GM crops** that have been certified as organic
- Environmentalists are concerned about the **reduction in biodiversity** for future generations, caused by **monocultures** of GM varieties
  - There is a theory that agricultural monocultures are **not sustainable** without heavy use of fertilisers
- Crops with less genetic diversity are more **vulnerable to extinction**
  - GM crops may become weeds or **invade the natural habitats** bordering the farmland
- Herbicide-resistance genes could transfer to weed plants resulting in "**superweeds**"
- GM crops that produce toxins may cause harm to non-target species like the Monarch butterflies
- The antibiotic-resistance genes that are commonly used as **marker genes** in genetic modification could transfer to pathogenic organisms that would then be untreatable with antibiotics - "**superbug**"
- Tampering with viral genomes could result in a completely **novel animal virus** that can affect humans or cause existing ones to become **more harmful** to the host
  - This is only an issue if the pathogens are able to escape the lab and enter the wild
- Over time **mutations may occur in the inserted genes** that cause them to have unwanted effects on organisms


Your notes

3.4.4 Cloning


Your notes

Cloning

- Clones are groups of genetically identical organisms, derived from a single original parent cell
- A cloned cell is a cell that is **genetically identical** to the cell that it originated from
- Sexual reproduction produces a **zygote** when gametes fuse
- In a single birth, this zygote is not cloned and will itself reproduce sexually as an adult
  - Identical twins are **clones of each other** as they are formed from one zygote splitting into two parts, which each develops into an embryo
- Clones form **naturally** and **artificially**
- The simple gardening technique of **taking plant cuttings** relies on cloning
- Other organisms are manipulated to **form multiple clones** when grown commercially eg. large-scale growth of crop plants, to ensure a uniform crop and good crop yields
- This ensures that **desirable characteristics** appear in the phenotypes of every organism

Cloning: Natural Methods


Your notes

- Many plant species and some animal species have **natural** methods of cloning
- Asexual reproduction is **much less common in animals** than in plants
- Some small animals reproduce asexually by **parthenogenesis** eg. aphids
- The other naturally occurring incidence of cloning in animals is **identical twins**

Natural cloning in plants

- Many methods of cloning **do not require seeds** as it is not sexual reproduction that is occurring, it is **asexual reproduction**

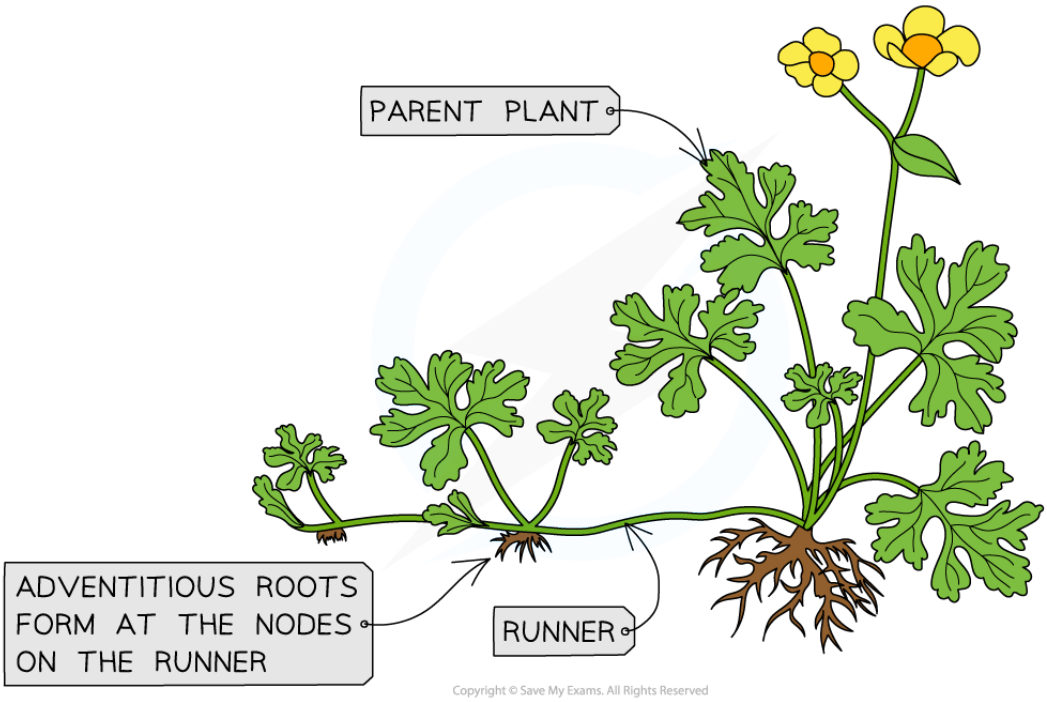
- A well as runners, plants can propagate asexually using **tubers, rhizomes**, **bulbs, suckers**, and **offsets**
- All modes of natural plant cloning contain **modified stems** that can generate **meristem tissue**
- Potato **tubers** are swollen modified roots that form **eyes** on their surface
  - Eyes can sprout new growth (called 'chitting')
  - The starch stored in the tuber fuels the early growth of the new plant

- Ginger forms **rhizomes**, a modified stem that grows horizontally underground
  - New growth stems from nodes in the rhizome, forming **new stems** and **adventitious roots**
  - The section used in cookery is the rhizome

- Onions and garlic form **bulbs** that can grow adventitious roots underground and leafy shoots above ground
- **Suckers** are growths that appear from the root systems of many trees and shrubs, which can provide meristematic tissue for vegetative propagation
  - Examples are **poplars, cherries** and **plums**

- **Offsets** are small, virtually complete daughter plants that have been asexually produced on the mother plant
  - Examples are **tulips** and **lilies**

Your notes



An example of natural cloning in plants with runners that form adventitious roots

Identical twins

- An **egg is fertilised by a sperm** as in a singleton birth
- This forms a **zygote**
- The single zygote undergoes a few cell cycles (mitotic divisions) to become an **embryo**
  - This is why identical twins are referred to as **monozygotic**
- At the embryo stage, the **embryo splits in two**; the exact causes of this kind of split are not well understood
- The two embryos that form are **identical** (have exactly the same genotype) and develop *in utero* (i.e. in the uterus) together
- The result is the birth of **identical offspring**, always of the same gender, with identical phenotypes
- Because **non-identical twins** are formed from separate eggs and sperm, they are **not clones**

Your notes

Identical twins are natural clones when a zygote splits into two parts and each part develops into a separate embryo

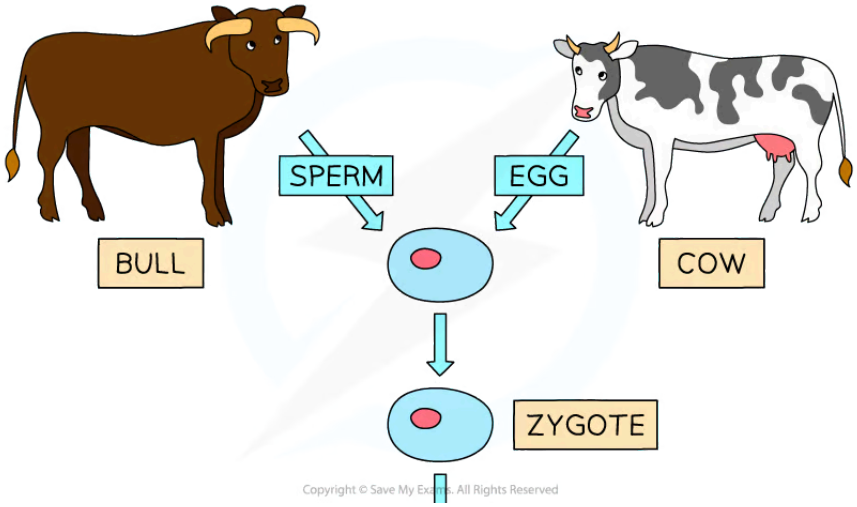
Examiner Tip

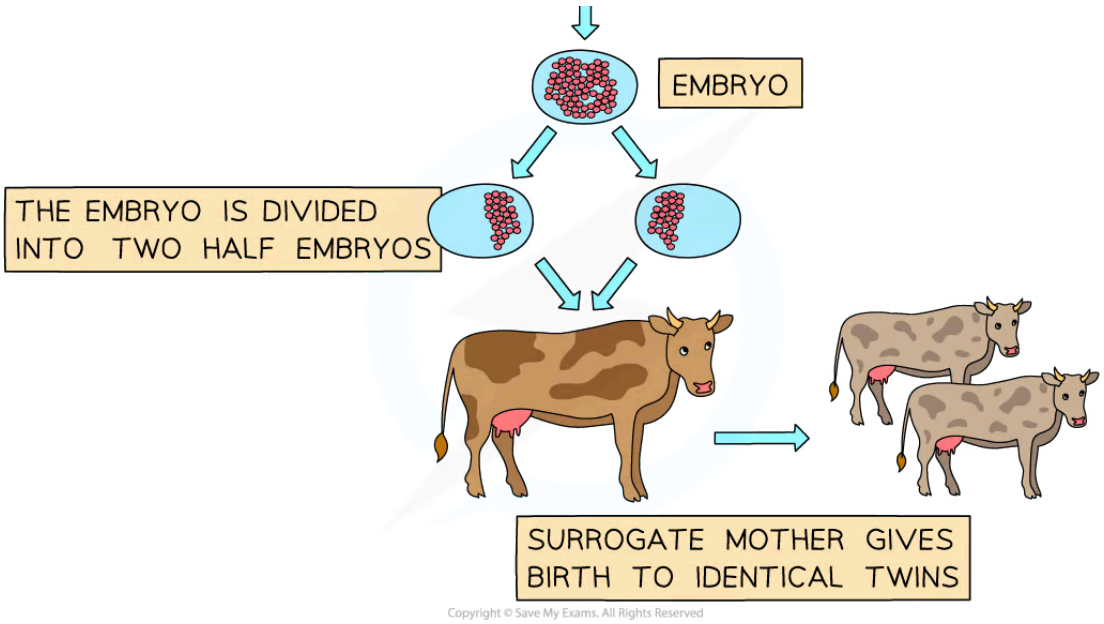
Although identical (monozygotic) twins share the same genome at the moment when the embryo splits, **identical twins are not clones** in the true sense of the word. Because **mutations** occur with every cell cycle, Twin A will possess slightly different DNA base sequences to Twin B at the time of birth. The older the twins get, the more their genomes become dissimilar as mutations accumulate. They will still look very alike throughout their lives unless there are large differences in their environments as they grow up.

Cloning: Animals

Your notes

- The process of **embryo twinning** (sometimes called splitting or fragmentation) produces offspring that are **clones of each other** but not of their parents
- It has been a **routine procedure** carried out to **boost yields of livestock** and **promote desirable characteristics** since the 1980s
- The key step is the **deliberate division of the embryo** into two half embryos
- Both halves contain cells that are **pluripotent**
  - The embryo is split at around the eight-cell stage
- These are then inserted into a **surrogate mother** for gestation and birth
- The surrogate gives birth to **identical twins**
- In some cases, embryos are split into **single identical cells**, each of which can be implanted into a **separate** surrogate mother animal
- Although embryo twinning guarantees desirable characteristics in the offspring, it is **not possible to predict how many offspring** will be produced **within a herd of livestock**, something of vital importance to a farmer





Embryo twinning of cattle by splitting the embryo

Therapeutic cloning

- This is a technique designed to use cloned cells to **replace dead or damaged cells** that cause a loss of function in an individual
- Embryos are **cloned as in reproductive cloning,** but the embryos are removed and subdivided
- Each individual embryo cell is pluripotent and can be cultured and **artificially differentiated** into any type of specialised cell
- In theory, **any specialised cell** can be derived by this method
  - Crucially, specialised **cells with the same genome as the sufferer** can be cloned and replaced
- An example is replacing specialised brain tissue in sufferers of **Parkinson's Disease**
- At present, there is **a lot of potential** for therapeutic cloning but **little clinical progress** has been made

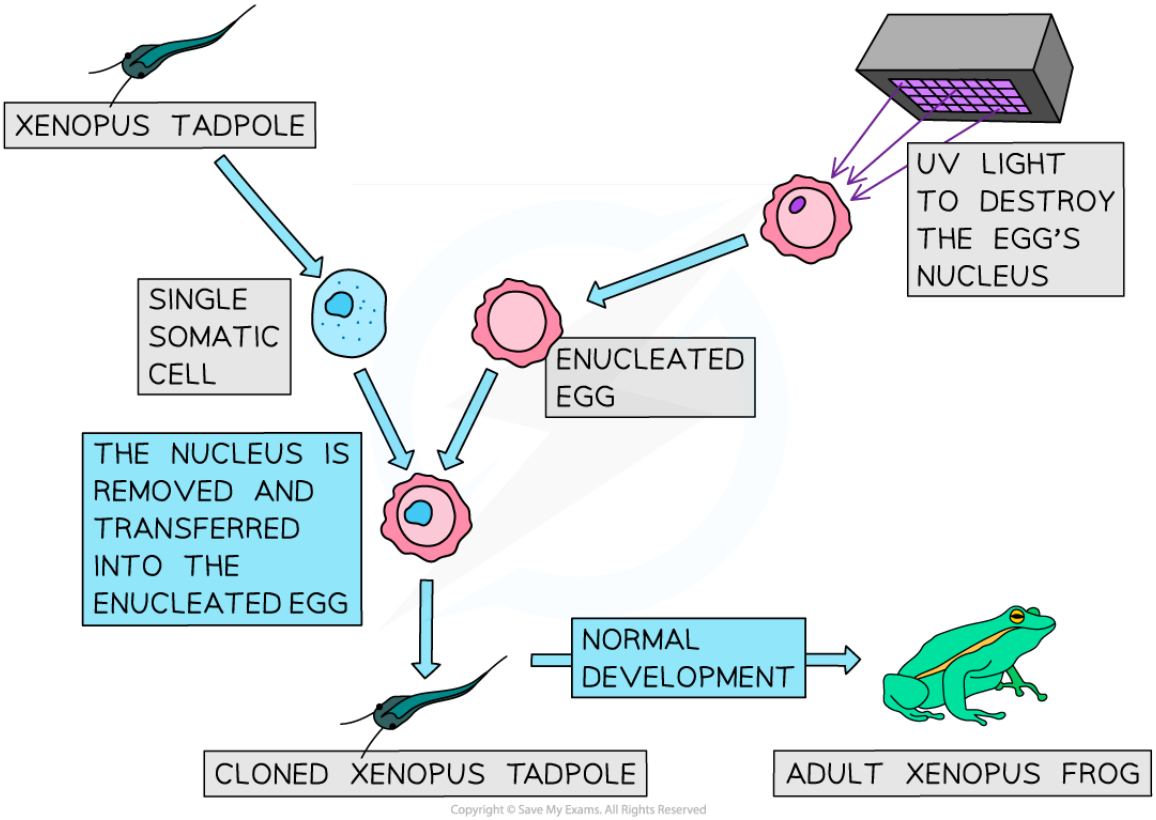
Your notes

Cloning: Using Differentiated Cells


Your notes

- Methods have been developed for cloning adult animals using **differentiated cells**
- It would be **desirable to clone a differentiated cell** because by then it would be easy to assess the organism's characteristics and whether any of its traits were desirable enough to clone
  - Pluripotent cells can develop into any specialised cell, but it is difficult to predict whether, once differentiated, they will display desirable characteristics
- Differentiated cells are more difficult to clone because **certain genes have been permanently switched off** (those genes will never be transcribed again) as the cell has developed its specialised role
- However, pioneering work on *Xenopus* (the African clawed frog) in the 1950s involved:
  - Removal of nuclei from *Xenopus* tadpoles' **somatic cells**
  - It was significant that the somatic cell was already **fully differentiated** eg. a skin cell
  - Insertion of these nuclei **into enucleated *Xenopus* egg cells** (eggs whose own nuclei had been destroyed by UV radiation treatment)
- This resulted in embryos that grew, divided and differentiated into **fully-functioning live tadpoles**, and ultimately, adult *Xenopus* frogs
- Whilst this work was successful, cloning mammals from differentiated cells proved much **more difficult**
- The work on *Xenopus* frogs **laid the platform** for the first mammal cloned by nuclear extraction
- It wasn't until the 1990s that the first large mammal, **Dolly the sheep,** was cloned successfully

Your notes



Cloning of* Xenopus *frogs by nuclear transfer from a differentiated cell

Somatic Cell Transfer

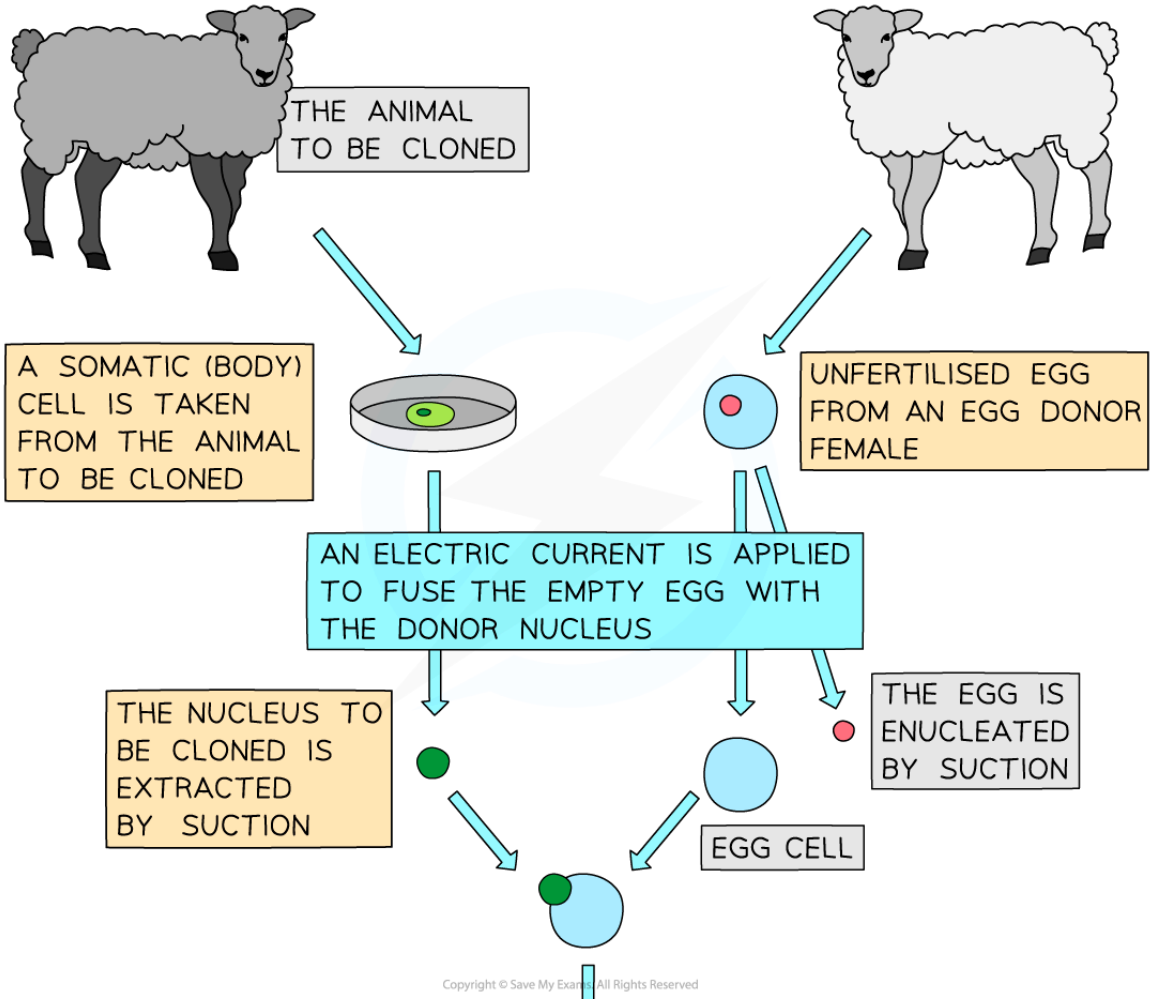

Your notes

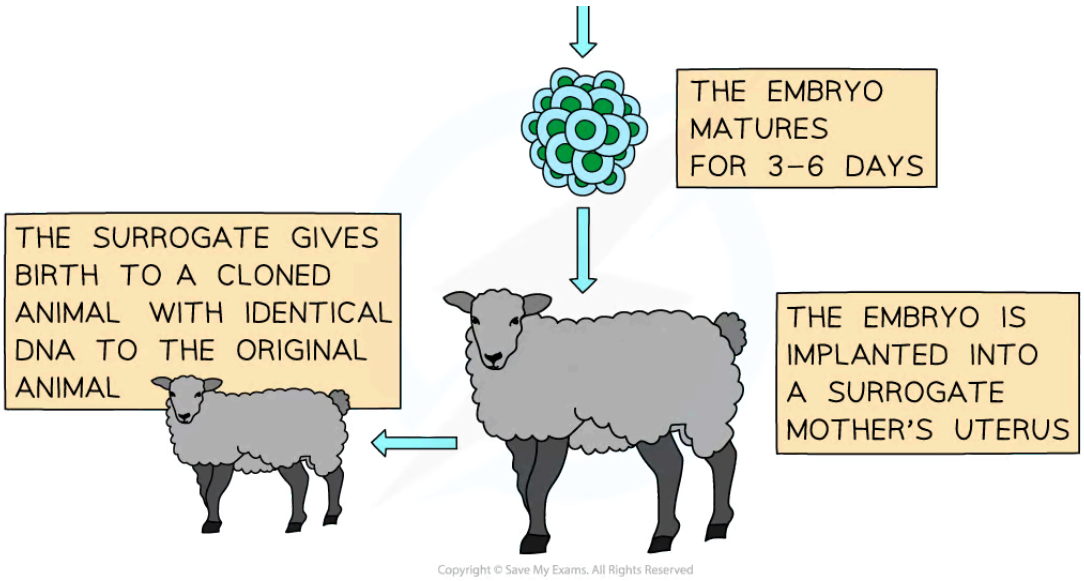
Production of cloned embryos produced by somatic cell nuclear transfer

- This is the method made famous by **Dolly the sheep**, cloned in Edinburgh, UK in 1996
- Its full name is **Somatic Cell Nuclear Transfer** (SCNT)
- Dolly made headlines as being the **first livestock animal to be created from a clone**
- Three separate animals are required:
  - The **animal being cloned** (by donating a cell)
  - The female to **donate an egg** cell
  - The **surrogate mother**

- How the procedure is carried out:
  - The animal to be cloned **donates a somatic (body) cell**
    - In Dolly's case, this was an udder cell
  - The egg cell is extracted from the egg donor and **enucleated** (its nucleus is removed by suction and discarded)
  - The nucleus from the udder cell is **injected into the enucleated egg** cell
  - The hybrid zygote cell is now treated to **encourage it to divide** by mitosis
  - The embryo is **implanted into the surrogate mother** for gestation and birth

Your notes





Reproductive cloning of animals by somatic cell nuclear transfer

Your notes

3.4.5 Skills: Genetic Modification & Biotechnology

Your notes

Cloning Plants

Design of an experiment to assess one factor affecting the rooting of stem-cuttings

- Stems can be cut so that **roots develop** from the cut end of the stem
- This is a way of **cloning plants artificially**, used routinely by gardeners and horticulturalists
- In most plants, a stem cut **below a node** is the ideal place to cut
  - A node is the position where leaves branch off the stem
- Leaves below this point are removed
- The bottom section of the stem is inserted into **compost** or **water**
  - Compost must be **sterilised** beforehand by heating
  - Compost should be **well watered** and **aerated**
  - Hormone rooting powder may assist the process of rooting
- A plastic bag with holes cut in it is used to cover the plant, to **prevent excessive water loss**
- Rooting takes a few weeks until the cutting is rooted independently in its soil
- The **success of rooting is variable** and can be tested by experimentation

Worked example

Design an experiment to assess the effect of adding hormone rooting powder to a plant cutting before rooting.

Step 1: Decide the independent variable

This is what I, as a scientist, alter. This is whether to use hormone rooting powder or not when planting out cuttings

Step 2: Decide the dependent variable

This is what I will measure at the end of the experiment. This will be the mass of root matter formed by the cuttings. The root matter will be removed using a sharp scalpel and weighed on an accurate lab balance

Step 3: How will the amount of root formation be measured?

This can involve cutting away all the root material and weighing it

Step 4: What variables should be kept constant for a valid investigation?

Species of plant, brand of compost used, mass of compost used, the sterilisation method of the compost used, pot size, length of cutting, approx leaf surface area present, light intensity, temperature, size/material of the plastic bag, size/number of holes in the plastic bag and time taken for the growth of plants

Step 5: How many different types of plants should be used?

A plant species should be chosen for rooting experiments that form roots readily in water or a solid medium. Basil plants (*Ocimum basilicum*) are readily available, inexpensive and form roots easily

Your notes

Step 6: How many cuttings should be used for each treatment

Ideally, three repeats (minimum) for each treatment. This allows repeats for the identification of anomalies and calculation of a reliable mean

Step 7: Draw out a blank results table to frame the results

This helps to refine the experimental design before lab work starts

Blank Results Table

Control variables	300g compost (sterilised) in a brown plastic pot (15cm height), 10cm cutting length, approx. 12 leaves, 25 °C, light from table lamp at 30cm distance, plastic bags 6×2cm cuts, 5 days growth time			
	Mass of root growth / g			
Independent variable	Cutting 1	Cutting 2	Cutting 3	Mean
Rooting powder used (approx 5g per cutting)				
No rooting powder used				

Step 8: Data processing

How will I process the data I generate to give meaningful conclusions?

Calculate a % change in the mean mass of root material from using no rooting powder to using rooting powder

Step 9: Improvements to the experiment

Drying the root material in an oven at 50°C to determine the dry mass of root material. This removes variation caused by possible fluctuations in root tissue's water content

Conduct more repeats to improve the reliability of the data

Perform experiments on different plants or by using a different brand of rooting powder/combinations of plant hormones

Your notes

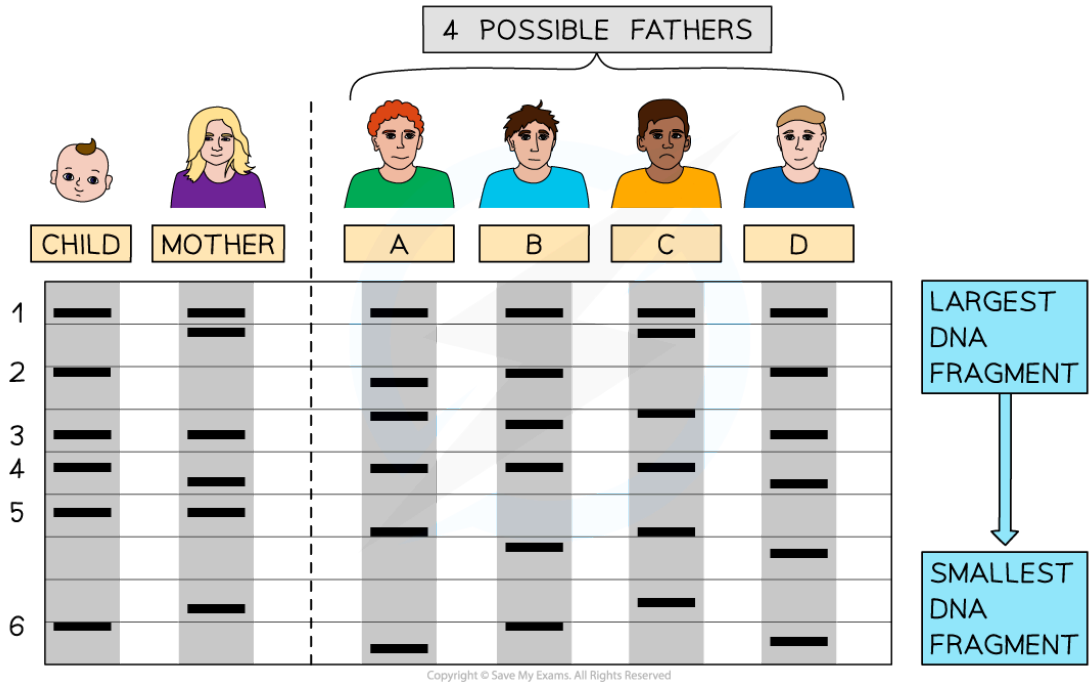
Analysis of DNA Profiles

Your notes

- In forensic investigations, samples of cells or bodily fluids containing DNA have to be taken from all **victims**, **witnesses** and **suspects**
- Care must be taken **not to mix up/contaminate** samples
  - Defence lawyers often seek to scrutinise the accuracy of DNA sampling because the identification of a sampling error can **invalidate evidence** and result in an **acquittal**
- The same pattern of bands in a specimen left at a crime scene and a possible suspect is often **enough to convict** that suspect
- In paternity investigations, analysis is more complicated because each band found in the child's DNA profile must be found either in the mother's or the father's DNA
- If a child displays a band that is **not displayed by the mother or the man who is presumed to be the father**, a different man must be that child's father (see page 3.4.2 DNA profiling)

Worked example

Paternity Investigation: Who's the Father? – Use the DNA profiles of all 6 people shown to work out who the child's father is



Remember, any band showing in the child's profile must be present in the mother **OR** father's profile, **OR** both. If not, that man is not the child's father.

Step 1: Look at the child's first DNA band (labelled 1)

The mother possesses this same band, so the child could have inherited that DNA from its mother. It is therefore of no benefit to look at whether any of the men possess that band

Step 2: Look at the child's second DNA band (labelled 2)

The mother does not possess this band, so the child must have inherited it from its father. Only men B and D possess this band, so men A and C are eliminated

Step 3: Look at the child's third DNA band (labelled 3)

As with band 1, the mother possesses this same band, so the child could have inherited that DNA from its mother. It is therefore of no benefit to look at whether any of the men possess that band

Step 4: Look at the child's fourth DNA band (labelled 4)

The mother does not possess this band, so the child must have inherited it from its father. Only men A, B and C possess this band, but A and C have already been eliminated

Step 5: Conclude that B is the father

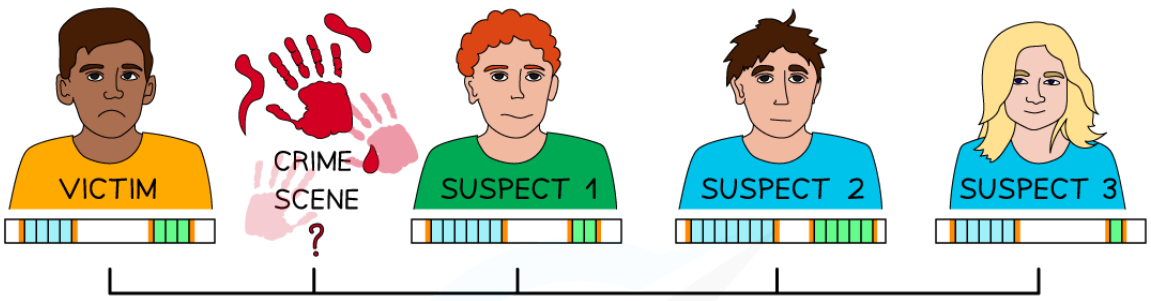
Step 6: Look for supporting evidence from band 6

The mother does not possess this band, and the only man who possesses it is B. **This reinforces the conclusion that Man B is the child's father**

Forensic Investigations


Your notes

Your notes



KNOWN LENGTHS

Control DNA	Victim	Crime scene	Suspects		
			1	2	3

Using DNA profiling in criminal investigations. Suspect 3 has the most fragments in common with the crime scene DNA so it is likely that Suspect 3 is the culprit.

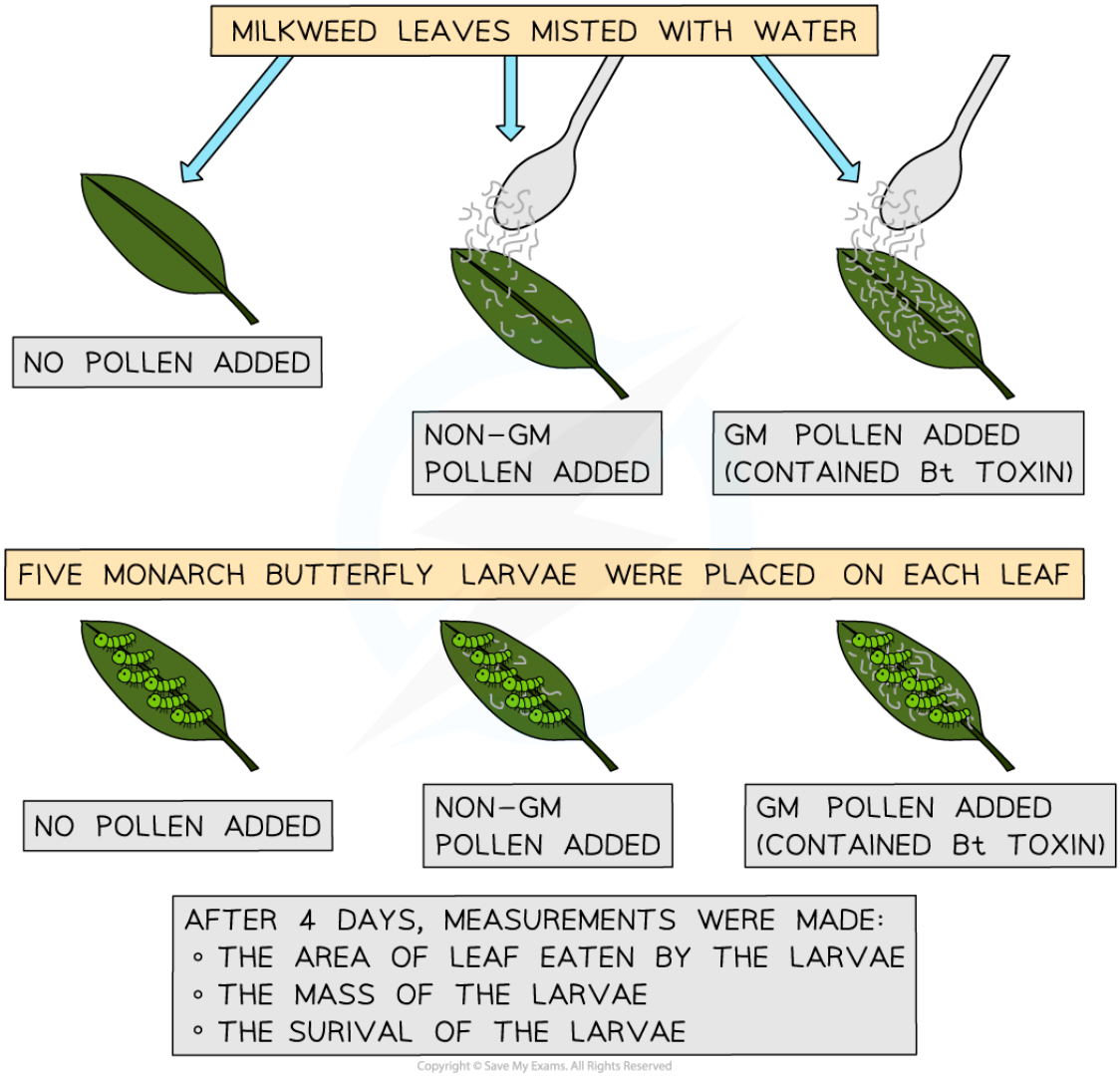
Assessing the Ecological Risks of GM Crops


Your notes

Analysis of data on risks to monarch butterflies of *Bt* crops

- The bacterium *Bacillus thuringiensis* (or *Bt* for short) produces **a natural protein toxin** that has insecticide properties
  - Many groups of insects are killed by this toxin including, **bees**, **flies**, **beetles** and **butterflies**
    - This causes considerable **collateral damage** to the surrounding ecosystem
- Farmers growing corn (maize) have to spray their crop with insecticide to prevent insect pests such as corn borers
- Corn has been genetically modified to express the *Bt* toxin gene in all its tissues including its pollen
  - This **improves yields** and **greatly reduces the need for crop spraying**, so has benefits to the farmers
- One species affected by *Bt* toxin is the **monarch butterfly** (*Danaus plexippus*)
  - *D. plexippus* larvae feed on milkweed that grows in the vicinity of corn crops
  - Milkweed that becomes dusted with pollen that is spread by the wind from transgenic corn can poison the monarch butterfly larvae, leading to a **reduction in numbers of the butterfly**
- This effect can be **investigated by experimentation** to examine the effects of growing transgenic *Bt* toxin corn

Your notes

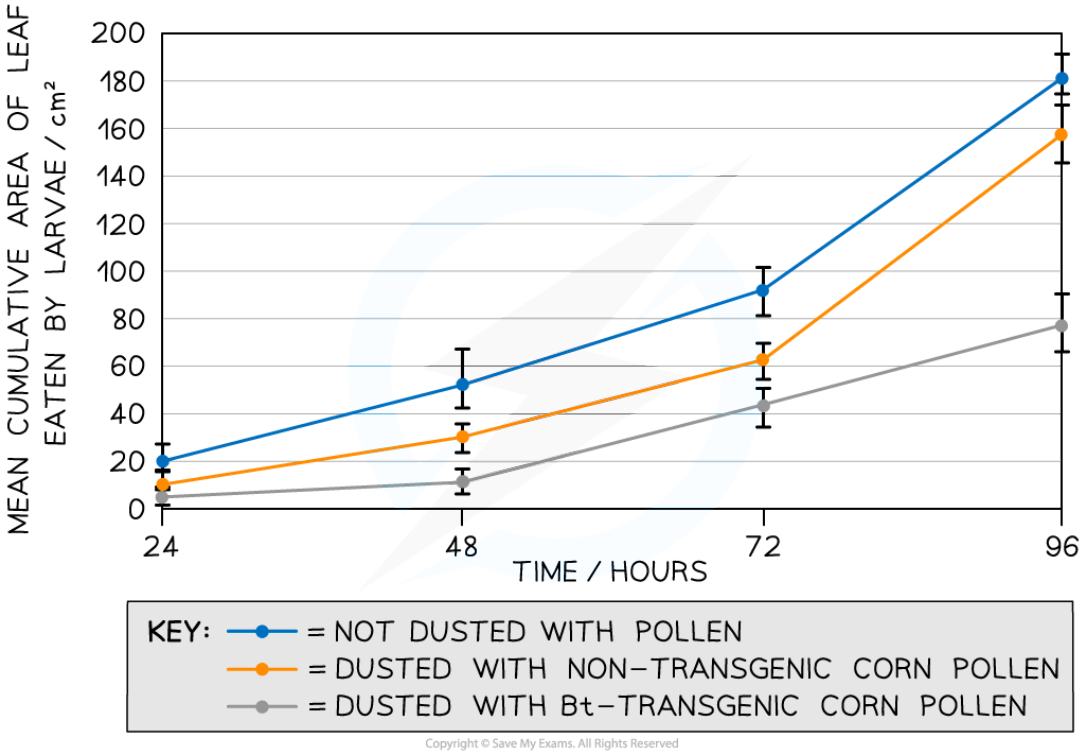


Experimental set-up to prove the effect of Bt toxin-containing pollen on monarch butterfly larvae

The Effect of Various Pollen Types on Monarch Butterfly Larvae Results Table

Your notes

Time / hours	Not dusted with pollen		Dusted with non-transgenic corn pollen		Dusteed with Bt-transgenic corn pollen	
	Area of leaf eaten / cm^3	Cumulative area of leaf eaten / cm^3	Area of leaf eaten / cm^3	Cumulative area of leaf eaten / cm^3	Area of leaf eaten / cm^3	Cumulative area of leaf eaten / cm^3
24						
48						
72						
96						



A graph showing the results from the experiment

Results summary

- The results showed that the larvae not dusted with pollen ate more of the leaves
- Of the larvae dusted with pollen, the ones dusted with non-GM pollen ate more of the leaves
- The survival rate of the larvae dusted with GM pollen was the lowest of all three

- The mean mass of larvae at the end of the study was double for non-dusted larvae than for those dusted with GM pollen
- These data show a **strong link between the use of *Bt* toxin GM crops and collateral damage** to a neighbouring animal species

Considerations for experimental setup

- **Control variables** would be:
  - The size/age of the larvae at the beginning of the experiment
  - Same size/area of leaves used in each experiment
  - Same species of larvae/milkweed
  - Same temperature/illumination/growth medium
  - Same availability of water
- **Five repeats** were completed per experiment for reliability
- **Error bars** were constructed in the data to demonstrate a low likelihood that a link between GM crops and monarch butterfly populations could have occurred by chance

Examiner Tip

Drawing a **blank results table** before any experiment is a good idea for a few reasons:

1. It helps the scientist to design the correct size and scope of the experiment
2. It may prompt the scientist to perform an experimental variation that he/she hadn't thought of when setting up the initial investigation
3. It is neater and more systematic for recording experimental results; hands can get quite messy when conducting experiments, especially where plants and soil are involved!


Your notes