IB Biology DP

YOUR NOTES

3. Genetics

CONTENTS

YOUR NOTES

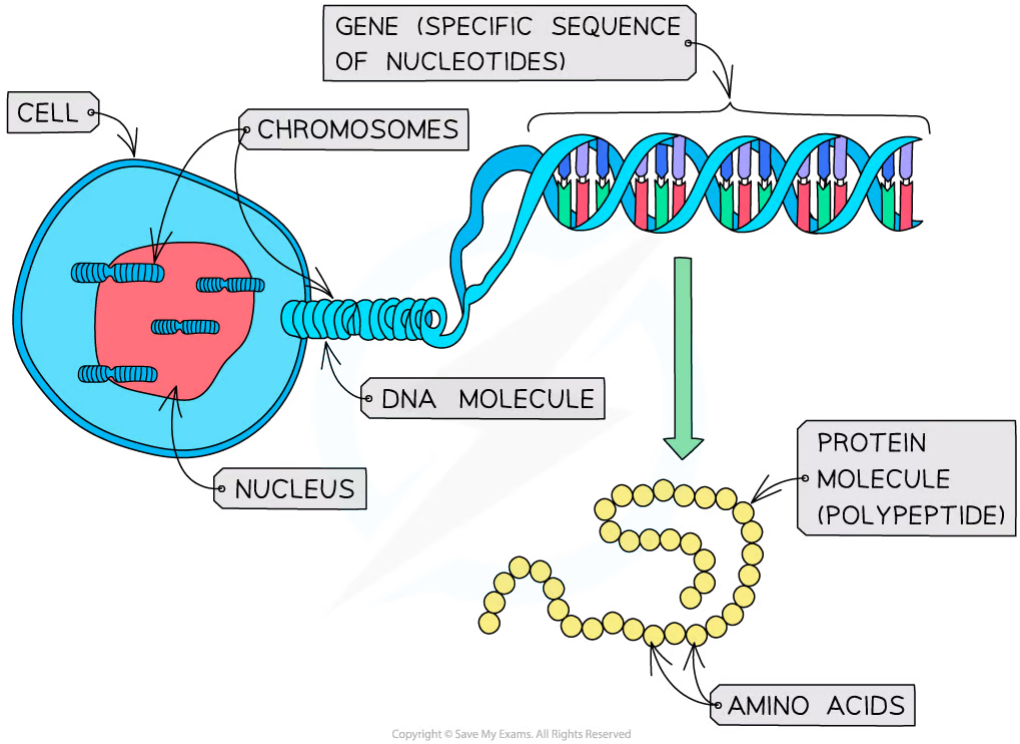
3.1 Genes & Chromosomes

3.1.1 Genes

Genes & Polypeptides

- A gene is a section or **length of DNA** that codes for a **polypeptide**
- Genes are **heritable factors** that influence specific **characteristics** (via the polypeptides produced)
  - **Characteristic** means a feature of an organism like **height** in pea plants or **blood group** in humans
  - **Heritable** means genes are factors that pass from parent to offspring during reproduction

- A gene occupies a **specific position** on a **chromosome**
- The gene for a particular characteristic is always found at the same position or **locus** (plural is **loci**) on a particular chromosome



A gene consists of a length of DNA found in the nucleus. This length of DNA causes a specific characteristic by coding for specific polypeptides.

YOUR NOTES

Exam Tip

Remember – each chromosome in a human cell nucleus contains one very long DNA molecule. This DNA molecule is made up of thousands of specific nucleotide sequences called genes that code for specific polypeptides.

Loci

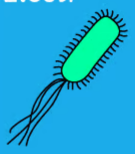
- A single chromosome contains **several hundred or thousands** of genes
  - Dependent on the length of the chromosome
- Through experiments and genetic mapping techniques, scientists were able to work out the specific **physical location of a gene on a chromosome**
- The location of a gene on a chromosome is known as its **locus** (the plural of locus is loci)
- Each gene occupies **a specific locus** so that the gene for a particular characteristic is always found at the same position on a particular chromosome

YOUR NOTES

Comparing the Number of Genes

- Species vary in the **number of genes** they have
- The number of genes a species has is **not related** to the size/complexity or even the sophistication of the organism
  - Because genes can vary in length
- **Counting the exact number of genes in a species is difficult**, so you may see conflicting numbers in different sources
- **Humans** have around 20,000 genes
- Dogs have 19,000 genes, which is **less than humans**
- A **water flea** has **more than a human** with 31,000 genes
- *E. coli*, a **bacterium**, has only 4,300 genes
- A rice **plant** has 41,500 genes

Comparing the Number of Genes between Different Organisms Table

Organism	Human 	Dog 	Water flea 	Bacterium E.coli 	Rice plant 
Approximate number of genes	20,000	19,000	31,000	4300	41,500

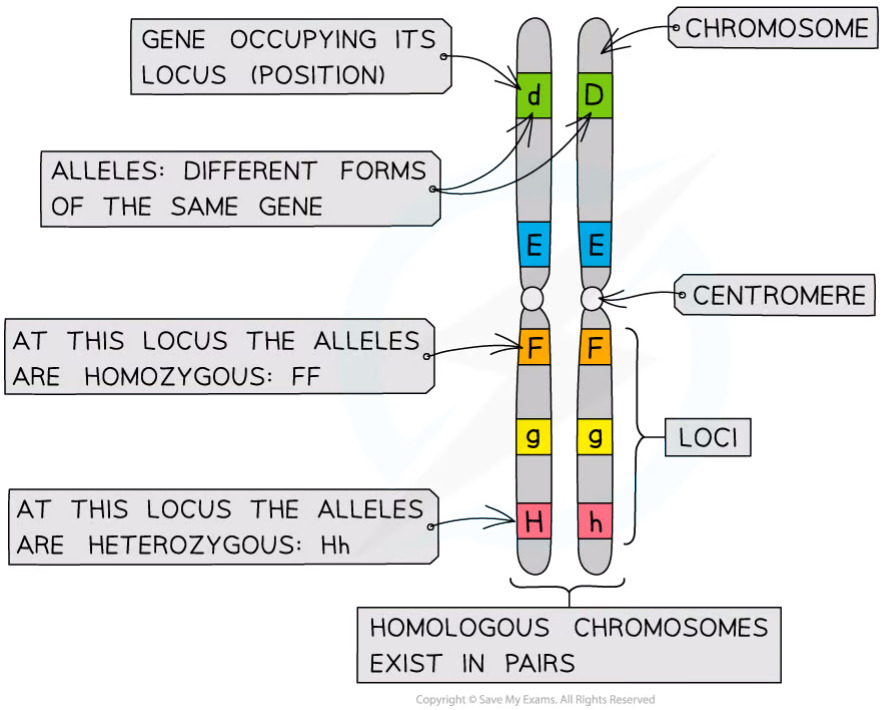
Exam Tip

For the comparison of the number of genes you need to know at least one plant and one bacterium, and at least one species with **more than humans** and one species with **fewer genes than humans**.The "number of genes" should not be referred to or confused with "genome size" as this term is used for the total amount of DNA (usually measured in the number of base pairs). Much of a eukaryotic species' genome does not code for polypeptides.

YOUR NOTES

Alleles

- A gene codes for a specific polypeptide that can affect a specific trait or characteristic in an organism
  - Eg. blood type
- **Alternative forms of a gene** can exist, these various specific forms are called **alleles**
  - Note that although alleles are different forms of the same gene, they all still occupy the same locus on the chromosome
  - New alleles occur through **mutations**
- **Multiple alleles** can exist for a gene that determines a specific trait
  - Each allele results in a different variation of that trait
    - Eg. blood types A, B, AB and O
- The chromosomes of eukaryotic cells occur in **homologous pairs** (there are two copies of each chromosome, one copy inherited from each parent) which means that cells have **two copies of every gene**
  - As a result, a cell possesses **two alleles of every gene** within its nucleus
  - When the two alleles at a locus are the **same/identical** they are described as **homozygous**
  - When the two alleles at a locus are **different** they are described as **heterozygous**



Chromosomes showing genes, loci and alleles

Differences between Alleles

YOUR NOTES

- Alleles differ from each other by **one or only a few bases**
- Even a very small change in base sequence can bring about a large effect in gene function, with a large knock-on effect on the **phenotype**
- Even though different alleles of a gene have slightly **different base sequences,** they still occupy the **same locus** on the chromosome
- Since the Human Genome Project, **sophisticated techniques** can analyse different alleles
- These techniques are becoming **faster**, **more accurate** and **more accessible** to individuals
    - Comparable sequences can be analysed down to individual bases to determine **evolutionary relationships**
    - The more differences in base sequence, the further apart two species are in evolutionary terms
- The exact positions where bases differ between alleles are called **SNPs** or **snips** (Single Nucleotide Polymorphisms)
    - An allele **can have several snips** but still only **differ by a few bases** from its other allele

Exam Tip

Use the term **allele** wherever possible in written answers, as it's always a more precise term than **gene**.

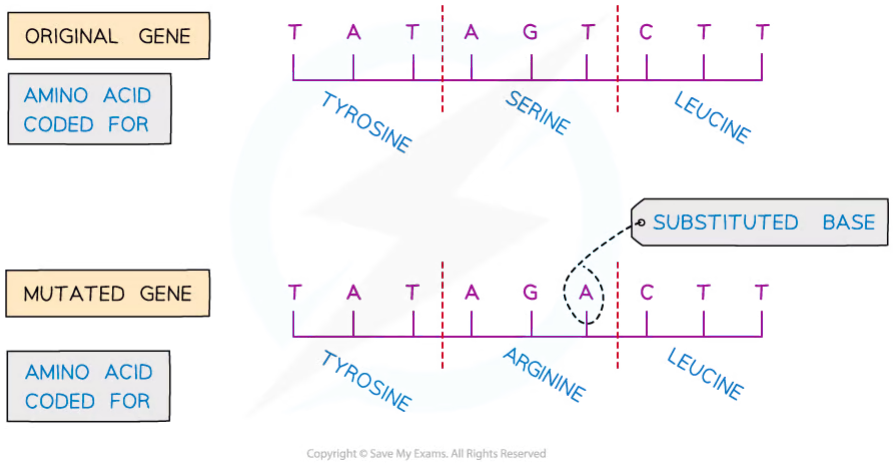
YOUR NOTES

Mutation

- A **gene mutation** is a change in the sequence of base pairs in a DNA molecule; this may result in a new allele
  - Mutations occur **all the time** and **at random**
  - There are certain points in the cell cycle when mutations are more likely to occur, for example, **copying errors** when DNA is being replicated (S phase of interphase)
- As the DNA base sequence determines the sequence of amino acids that make up a polypeptide, **mutations in a gene** can sometimes lead to a **change in the polypeptide** that the gene codes for
- Most mutations are **harmful** or **neutral** (have no effect) but some can be **beneficial**
- **Inheritance** of mutations:
  - Mutations present in normal body cells are **not inherited**, they are eliminated from the population once those cells die
  - Mutations within **gametes** are inherited by offspring, possibly causing genetic disease

Substitution mutations

- A mutation that occurs when a base in the DNA sequence **is randomly swapped** for a different base is known as a **substitution mutation**
- A substitution mutation will only change the amino acid for the triplet (group of three consecutive bases) where the mutation occurs; it will **not have a knock-on effect** further along the gene/polypeptide



An example of a substitution mutation altering the sequence of amino acids in the polypeptide

Exam Tip

You don't need to know about deletions, insertions and frameshift mutations - just **substitution** mutations!

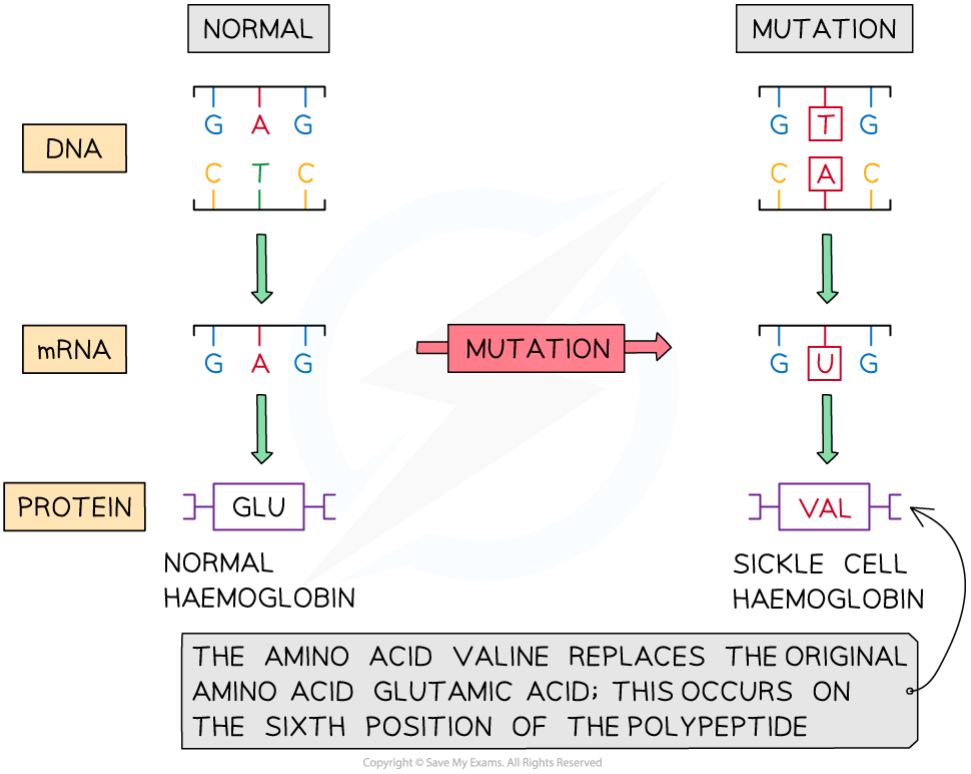
YOUR NOTES

Sickle Cell Anaemia

- A small change to a gene can have **serious consequences** for an organism
- Sickle cell anaemia is a genetic disease caused by **a single base substitution mutation** within the gene (*Hb*) that codes for the alpha-globin polypeptide in haemoglobin
  - Most humans have the normal allele **Hb^A**

The mutation that occurs

- Within the haemoglobin gene, the base thymine (T) is **replaced by the base adenine** (A). This causes the DNA triplet G**A**G to mutate to G**T**G
- The mutated DNA codon GTG is transcribed into the **mRNA codon GUG**, instead of GAG
- During translation the amino acid **valine** (VAL) replaces the original amino acid **glutamic acid** (GLU); this occurs on the **sixth position** of the polypeptide
- The slightly different polypeptide results in a new allele, **Hb^S**



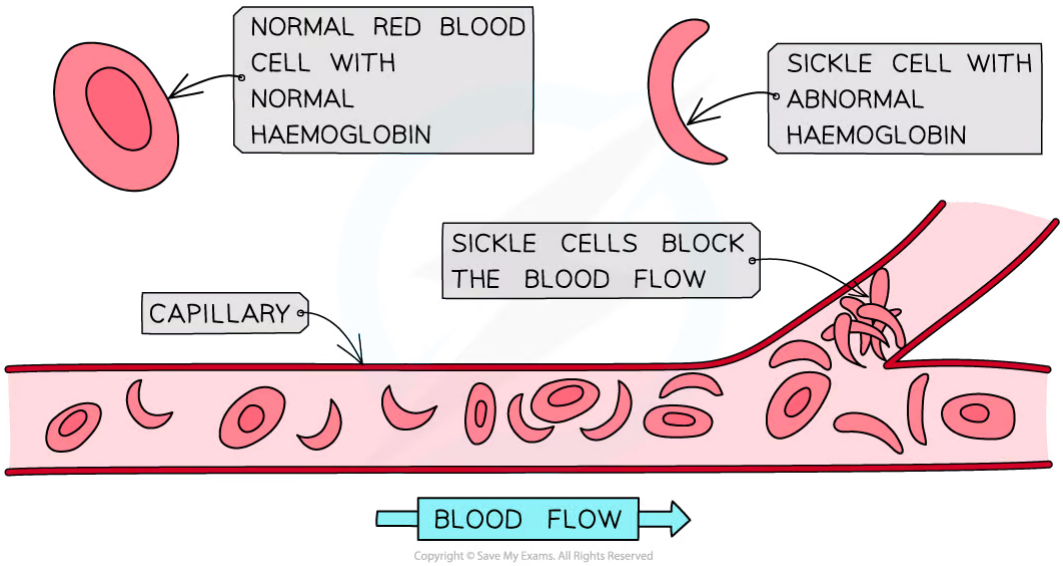
A base substitution on the DNA molecule results in a change in the amino acid at position 6 of the haemoglobin polypeptide, altering the overall structure and function of the protein

The effects

- The protein haemoglobin **S** is produced instead of haemoglobin A; this causes a **distortion in the shape of the red blood cells** into **sickle shapes**
- Sickle-shaped red blood cells:
  - Have a **limited oxygen-carrying capacity**
  - **Block the capillaries** limiting the flow of normal red blood cells
- People with sickle cell anaemia suffer from **acute pain**, **fatigue** and **anaemia**

YOUR NOTES

- There is a **correlation** between **sickle cell anaemia** and **malaria**
  - In areas with increased malaria cases, there is an increased frequency of sickle cell alleles



Normal red blood cells and sickle cell blood cells. The sickle cells cause a blockage in the capillary, restricting blood flow.

YOUR NOTES

Genome

- The **total of all the genetic information in an organism** is called the **genome** of the organism
- This is a **complete set of genes** present within every cell of an organism
- This includes **all genes** as well as non-coding DNA sequences
- **Mitochondrial DNA** and **chloroplast DNA** are included in the genome
- In a prokaryote cell, **plasmid** DNA is included in its genome

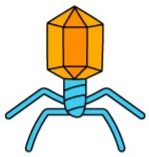
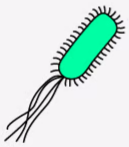
Comparing Genome Size

YOUR NOTES

- Advances in technology have allowed scientists to **write the whole sequence** of the genes within an organism's genome
- Genome-wide comparisons can now be made **between individuals** and **between species**
- Sequencing projects have read the **genomes of a wide range of organisms** from bacteria to humans
- Genome sizes can differ in different organisms:
  - Viruses and bacteria tend to have **very small genomes**
  - Prokaryotes tend to have smaller genomes than eukaryotes
  - The size of **plant genomes can vary widely**

Comparing the Genome Size of Different Organisms Table

YOUR NOTES

Organism	Common names	Genom Size (in million base pairs)
T2 Phage 	Virus that attacks E.coli	0.17
E. coli 	Bacterium	5
Drosophila melanogaster 	Fruit Fly	140
Homo sapiens 	Human	3000
Paris japonica 	Woodland Plant	150,000

Human Genome Project

YOUR NOTES

- The Human Genome Project (HGP) was an **international**, **collaborative, research programme** to sequence the entire human genome
- The work began in 1990, was **publicly funded**, and **shared** among more than 200 laboratories around the world, avoiding duplication of effort
  - Different labs sequenced different chromosomes
- DNA samples were taken from multiple people around the world, sequenced, and used to create a **reference genome**
- Because of rapid improvements in base sequencing technology the project finished ahead of time and was **published in April 2003**
- The finished genome was over **3 billion base pairs long** but contained only about 25,000 genes
- The HGP discovered new data about **non-coding DNA**, suggesting that it plays an active role in the cell, and that it isn't just 'junk' DNA
- The sequence of the DNA is stored in databases available to anyone on the Internet
- At the same time as the HGP, teams of scientists set about the **sequencing of the DNA of other organisms**. This included the human gut bacterium, *E. coli*, the fruit fly and the mouse. Since then, more than 30 non-human genomes have been sequenced

Applications of the Human Genome Project

- Three key impacts of the HGP include:
  - **How many individual genes** we have and how they work
  - Locating the **cause of genetic disorders**
  - Development of the new discipline of **bioinformatics** (the storage, manipulation and analysis of biological information via computer)
- The sequencing of the human genome has shown that **all humans share the vast majority (99.9%) of their base sequences**, but also that there are many **SNPs** that contribute to human diversity
- The information generated from the HGP has been used to tackle human health issues with the **end goal of finding cures for diseases**
- Scientists have noticed a correlation between **changes in specific genes** and the **likelihood of developing certain inherited diseases**
  - Several genes within the human genome have been linked to increased risk of certain **cancers**
  - There have also been specific genes linked to the development of **Alzheimer's disease**
- **Ethical, legal and social issues** are generated by the project

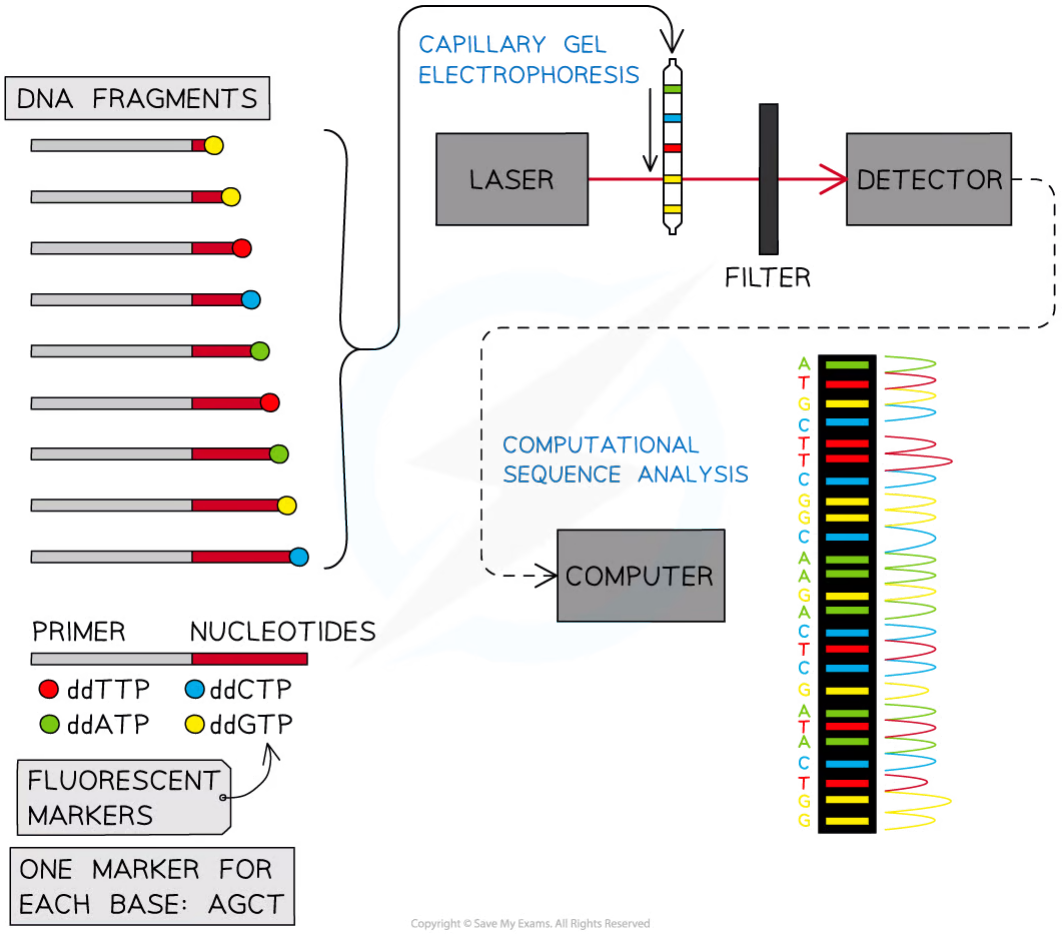
Genome Sequencing Techniques

YOUR NOTES

NOS: Developments in scientific research follow improvements in technology; gene sequencers, essentially lasers and optical detectors, are used for the sequencing of genes

- In scientific research, **critical developments often follow improvements in scientific apparatus**
- For example, distant objects in Space often remain **undiscovered** until a telescope (or some other piece of equipment) powerful enough to detect them is developed
- The fact that scientific research is often held back by a lack of sufficiently powerful or precise apparatus is a problem that will continue into the future
- In some ways, this is very exciting, as it suggests that our scientific knowledge and understanding of the universe will continue to expand as new scientific techniques and technologies are developed
- Investigations such as the Human Genome Project are **dependent on the use of powerful computers** and **improvements in technology** to store and analyse vast quantities of **data**
- To sequence a genome:
  - The entire genome is **broken up into manageable pieces** and then the **fragments are separated** so that they can be sequenced individually
  - Single-stranded copies are made
  - Nucleotides are each tagged with a differently **coloured fluorescent marker,** one for each base, adenine, cytosine, guanine and thymine
  - Samples are **separated according to length**, by **capillary electrophoresis machine**. This procedure is **very high resolution** and distinguishes DNA fragments that differ in size by only a single nucleotide
  - After separation, a **laser beam makes the fluorescent markers fluoresce**
  - Then an **optical detector** linked to a **computer** deduces the base sequence from the sequence of colours detected
- This process highlights the use of a database to **determine differences in the base sequence of a gene** in two species

YOUR NOTES



Gene sequencing is made possible by the use of optical laser technology, optical detectors, and computers

YOUR NOTES

Prokaryotic Chromosomes

- The DNA in prokaryotic cells is significantly different from the DNA found in eukaryotic cells
- Prokaryotes **do not contain a nucleus** therefore the DNA is located in the cytoplasm of the cell
- Prokaryotic DNA consists of a **single, circular chromosome**
  - It is sometimes referred to as a **nucleoid**
- The DNA within prokaryotic cells is **not associated with any proteins**
  - Prokaryotic DNA is sometimes referred to as **naked**
  - Eukaryotic DNA associates with **histone** proteins

Plasmids

- Prokaryotes also usually have one or more **plasmids**
  - Most eukaryotes do not contain plasmids
  - **Yeast** are the only types of eukaryotes that contain plasmids similar to those in prokaryotes
- Plasmids are **very small circular DNA molecules**
  - They usually **only contain a few genes**
  - They are **short**, typically 100,000 base pairs in length
- They are **more accessible for proteins** required for gene expression and therefore contain genes that are required often, quickly and/or in emergencies
- Plasmids can sometimes be **passed 'sideways' from one cell to another**, outside of the normal inheritance pattern during cell division
- They can also be used as a **vector** during genetic engineering to transfer DNA between species

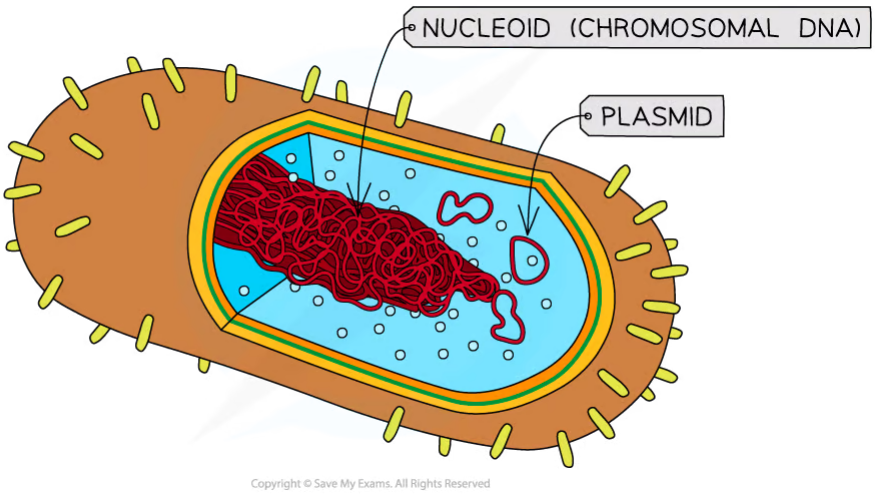


Image showing the arrangement of DNA within a prokaryotic cell

Autoradiography

YOUR NOTES

NOS: Developments in scientific research follow improvements in techniques; autoradiography was used to establish the length of DNA molecules in chromosomes

- In scientific research, **critical developments often follow improvements in scientific apparatus**
  - For example, distant objects in Space often remain undiscovered until a telescope (or some other piece of equipment) powerful enough to detect them is developed
- The fact that scientific research is often held back by a lack of **sufficiently powerful or precise apparatus** is a problem that will continue into the **future**
- In some ways, this is very exciting, as it suggests that our scientific knowledge and understanding of the universe will **continue to expand** as new scientific techniques and technologies are developed
- **Autoradiography** is a technique used to **study DNA** by **labelling** it using **radioactive isotopes**
  - These isotopes were fed to *E. coli* bacteria which incorporated them into their DNA
- When exposed to a **photographic film** the radioactive isotopes caused the film to become developed, resulting in an **image of the DNA** being produced
- In order to do this scientists use a **radioactive version of the DNA base thymine**, due to the fact this isn't found in RNA
  - The reason why the thymine is radioactive is because it contains **tritium**, a radioactive isotope of hydrogen
  - If scientists want to study RNA (for example, during the process of transcription) they can **use a radioactive version of uracil instead**, as this is not found in DNA
- This technique can be used to study DNA in both eukaryotes and prokaryotes
- In the past, this technique has been used to **make new discoveries about the length and shape of DNA in different organisms**

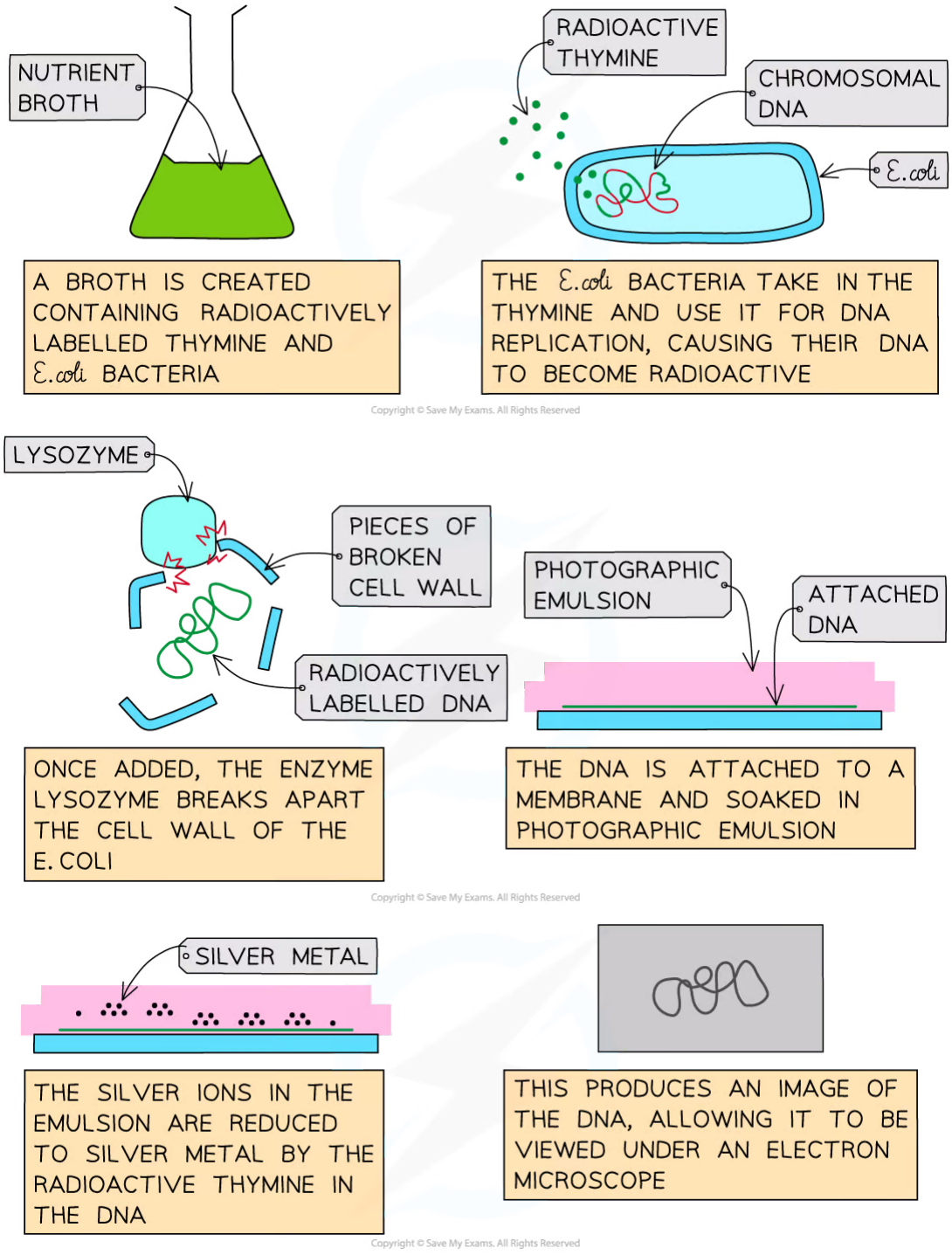
Cairns' Experimental Technique

YOUR NOTES

Cairns' technique for measuring the length of DNA molecules by autoradiography

- John Cairns was a scientist working in the field of molecular genetics and cancer research in the 1960s
  - During this time he pioneered a technique of using **autoradiography** to **study the DNA of *E. coli* to determine its length and shape**
- The method that Cairns used is as follows:
  - He first kept the *E. coli* bacteria in a nutrient broth containing a tritiated thymidine which is a **radioactive version of the DNA base thymine** attached to a deoxyribose sugar
  - The *E. coli* bacteria incorporated these bases into their DNA during **replication**. This meant that after several generations the DNA was fully radioactive
  - He then **lysed the cells** using an enzyme called **lysozyme**, breaking apart the **cell walls**, which allowed the DNA to be accessed
  - The DNA was fixed into position onto a membrane
  - The membrane was **submerged in a photographic emulsion** containing silver ions (Ag^+) for two months
  - When the silver ions were exposed to the radioactive DNA, the ions were reduced to silver metal. The grains of silver metal caused **visible black dots** to appear in the **photographic emulsion**
  - Once this emulsion had been developed it could be viewed under an **electron microscope** and the length and shape of DNA could be studied
- By using this technique Cairns made many important discoveries
  - He found that *E. coli* contains a **single**, **circular chromosome of DNA**
  - He also measured the length of the circular chromosome to be **1100µm long** (550 times bigger than the *E. coli* cell itself)
  - Cairns later went on to make important discoveries about the method of **DNA replication** in prokaryotes using this same technique

YOUR NOTES

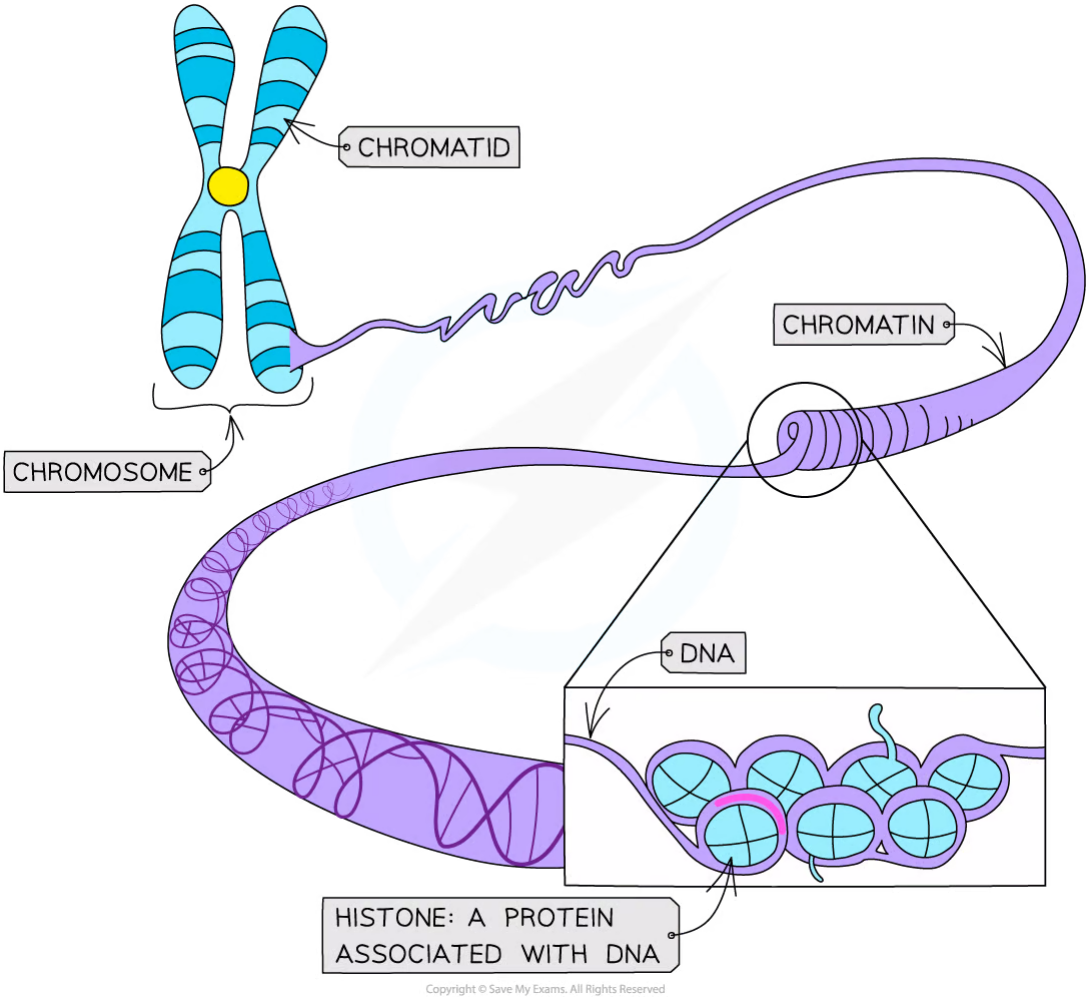


The Cairns' technique of using autoradiography to view DNA

YOUR NOTES

Eukaryotic Chromosomes

- Chromosomes in eukaryotic cells are **one, very long** DNA molecule associated with proteins
  - The main proteins present are the large, positively charged globular proteins called **histones**, their role is to **organise** and **wrap** the DNA tightly so that it fits into the nucleus
  - The other proteins are **enzymes** used in copying and repairing the DNA
- The tightly coiled combination of DNA and proteins is called **chromatin** – this is what chromosomes are made of



DNA is coiled around histone proteins to form chromatin

The replication of chromosomes

- During **interphase** (the period before mitosis) the DNA replicates to create **two identical strands of DNA called chromatids**, joined together by a narrow region called the **centromere**

YOUR NOTES

- The two chromatids that make up the double structure of a chromosome are known as **'sister chromatids'**
- It is important that the **sister chromatids** are **identical** in order to produce **genetically identical** daughter cells via mitosis
  - During **anaphase** of **mitosis** one chromatid ends up in one daughter cell while the other chromatid ends up in the other daughter cell
  - After the centromere is split apart at the start of anaphase the chromatids are referred to as individual **chromosomes** again

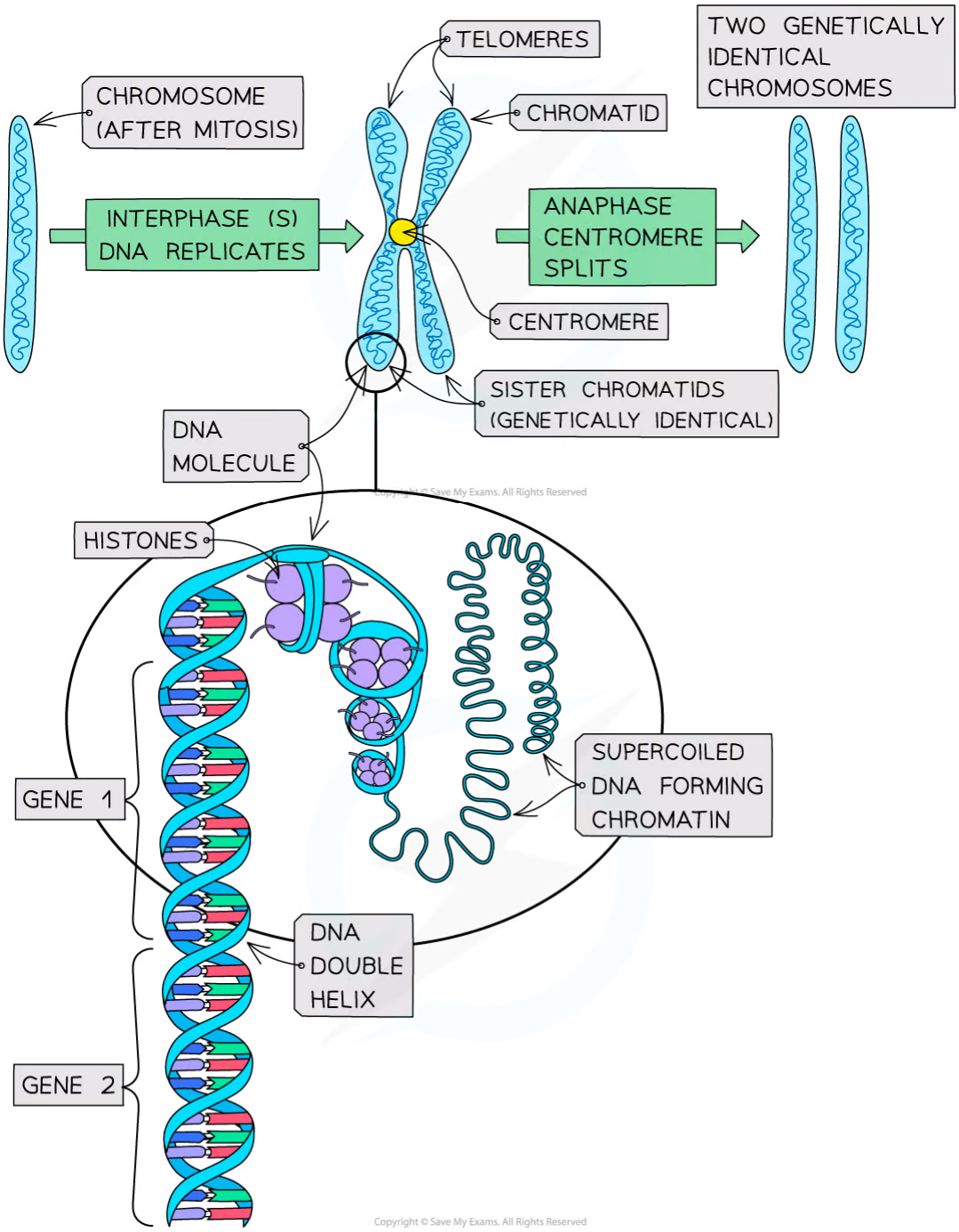


Diagram illustrating the structure of a chromosome at different stages of mitosis

YOUR NOTES

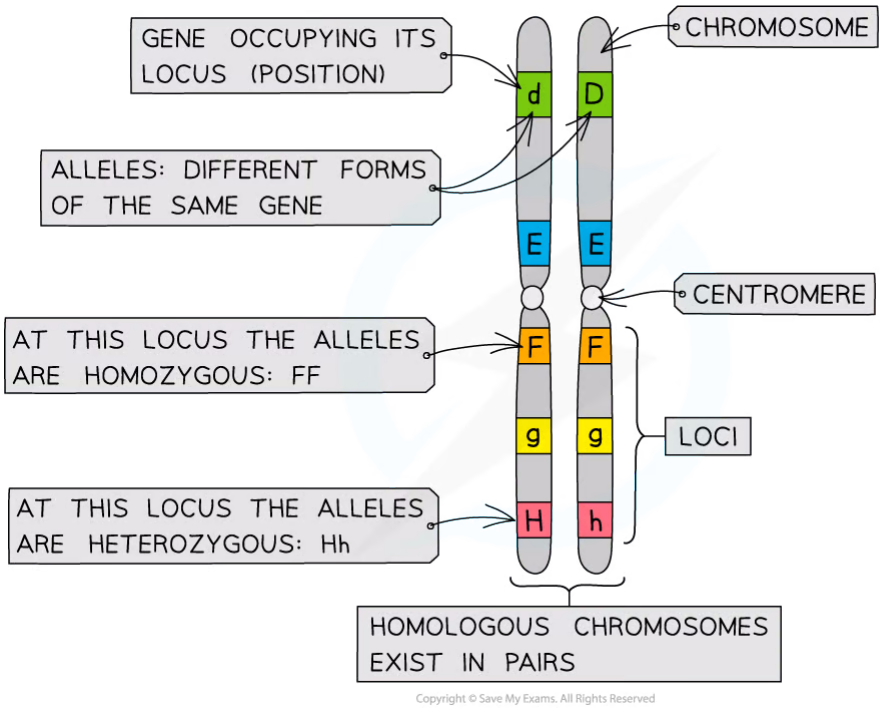
Exam Tip

It is important to distinguish between the terms chromatid, sister chromatids and chromosomes.

YOUR NOTES

Different Types of Chromosomes

- In a eukaryote species, there are different chromosomes that carry different genes
- During mitosis, chromosomes become denser by supercoiling, so **are easier to observe** than when they are in interphase
- Different types of chromosomes can be seen
  - They **differ in length** and the **position of the centromere**
- Humans have **23 types of chromosomes**
  - The largest one is numbered 1
  - The smallest is numbered 22
  - Pair 23 is the pair of sex chromosomes (XX or XY)
- Humans have between **20,000 and 25,000 genes** across all 46 chromosomes
- Specific **genes always appear at the same locus** (position) o**f a particular chromosome**, for example:
  - The SRY gene found on the Y chromosome causes the development of male genitalia such as the testes
  - The genes that determine eye colour are located on chromosome 15
  - The gene with a faulty version that leads to the disease cystic fibrosis is located on chromosome 7
- In other words, each chromosome type contains **specific genes arranged in a standard sequence**
  - This property allows for the exchange of genetic material between chromosomes during meiosis

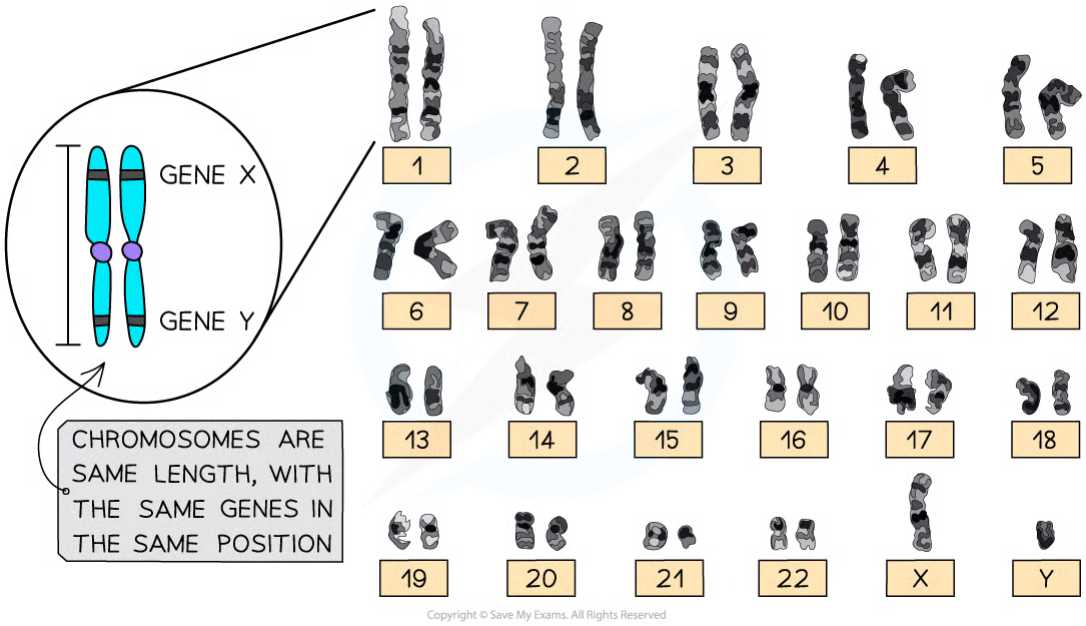


A pair of homologous chromosomes showing the loci of various alleles

YOUR NOTES

Homologous Chromosomes

- In **diploid** cells there are two complete sets of chromosomes in the nucleus
- **Homologous chromosomes**
  - Carry the **same genes at the same loci**
  - Are the **same shape**
  - Are **not usually identical** because they may be carrying **different alleles** to each other
- During fertilization, a diploid zygote is formed
  - In a zygote, one chromosome of each homologous pair comes from the female gamete and the other comes from the male gamete
- Having the same genes in the same **loci** helps homologous chromosomes line up alongside each other during Metaphase 1 of meiosis
- In photomicrographs, chromosomes are often grouped into their homologous pairs
  - These are shown in a picture format as a **karyogram**



Human karyogram showing homologous chromosomes

Exam Tip

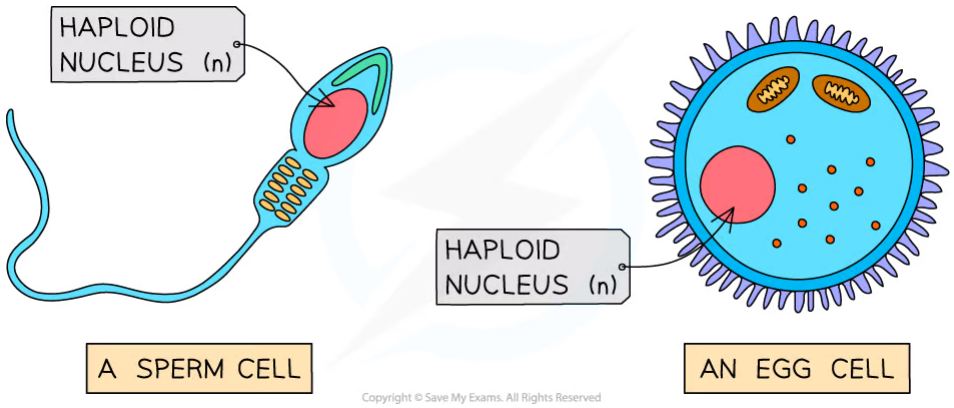
Although homologous pairs of chromosomes contain the same genes in the same order they don't necessarily carry the same alleles (form) of each gene!

YOUR NOTES

Haploid

Haploid nuclei have one chromosome of each pair

- Haploid cells contain **one complete set of chromosomes (n)**
  - In other words, they have **half** the number of chromosomes compared to normal body cells
  - Humans have **haploid** cells that contain **23 chromosomes** in their nucleus
    - One chromosome from each pair
    - n = 23
  - These haploid cells are called **gametes** and they are involved in sexual reproduction
  - In animals, they are the **female egg** and the **male sperm**



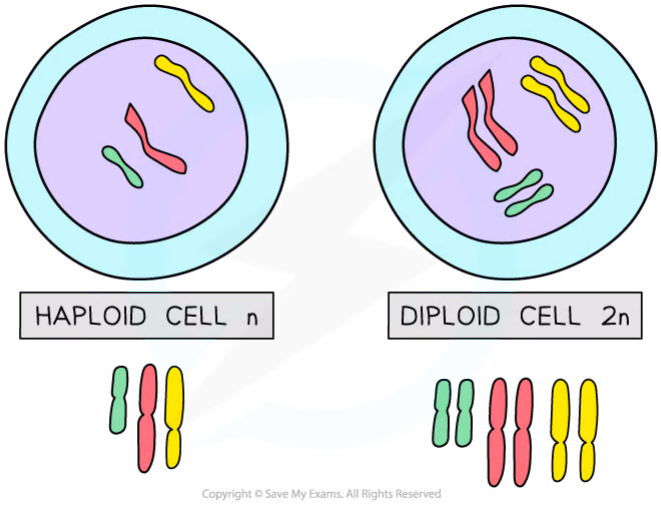
Gametes are haploid cells

Diploid

YOUR NOTES

Diploid nuclei have pairs of homologous chromosomes

- A **diploid** cell is a cell that contains **two complete sets of chromosomes (2n)**
- These chromosomes contain **all the DNA necessary** for protein synthesis and cell function
- A diploid cell (**zygote**) is formed from the **fusion of two haploid gametes** at fertilisation
- Nearly all cells in the human body are **diploid** with 23 **pairs** (46 in total) of chromosomes in their nucleus
  - Red blood cells are an exception to this rule because they do not contain a nucleus
- **2n = 46** in humans
- Having two alleles gives **some protection from harmful mutations** that are **recessive**
  - There is a copy of the correctly-functioning allele still present
- **Hybrid vigour** is often observed in individuals with **two different alleles**, and can be seen as strong growth and general good health



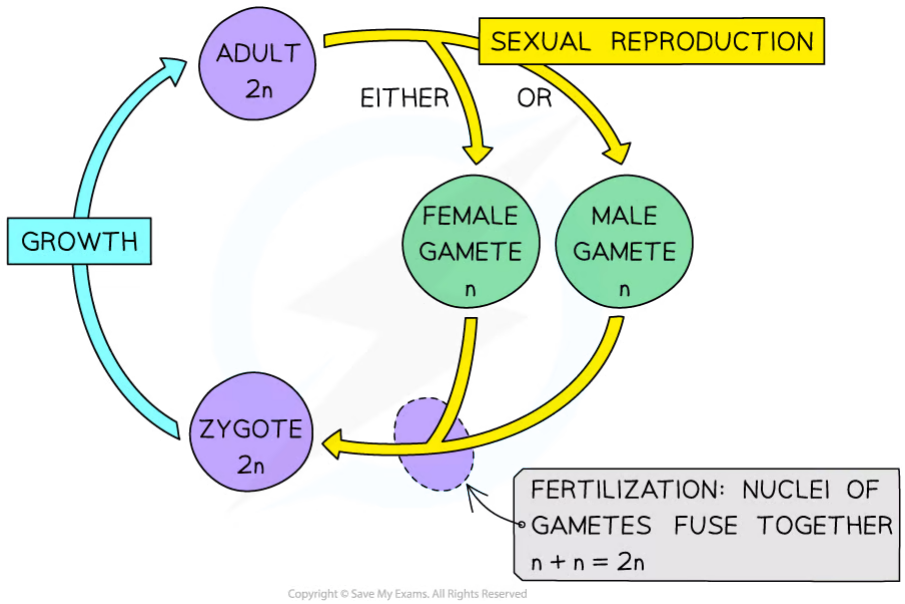
Haploid (n) and Diploid (2n) cells

YOUR NOTES

Number of Chromosomes

The number of chromosomes is a characteristic feature of members of a species

- During fertilisation the **nuclei of gametes fuse together** to form the **nucleus of the zygote**
- Both gametes must contain **the same number of chromosomes** in order for the zygote to be **viable**. If a zygote has too many or too few chromosomes it may not survive
- For a diploid zygote this means that the **gametes must be haploid**
  - n + n = 2n
- Meiosis produces haploid gametes during sexual reproduction
- The first cell division of meiosis is a **reduction division** (reduces the number of chromosomes)
  - This is a **nuclear division that reduces the chromosome number** of a cell
  - In humans, the chromosome number is reduced from 46 (diploid) to 23 (haploid)
- The reduction in chromosome number during meiosis ensures the gametes formed are haploid
- Sometimes during evolution, there can be **a change in the number of chromosomes** a species has; these events are very rare
  - Bread wheat (*Triticum aestivum*) is a species with **six** sets of chromosomes (6n)
  - These changes didn't occur randomly but were intentionally bred by humans to produce ideal characteristics in the bread wheat



The maintenance of chromosome number through reduction division in a mammalian life cycle

YOUR NOTES

Comparison of Chromosome Numbers

Comparison of diploid chromosome numbers of humans, chimpanzees, dogs, rice and horse threadworm

- These are their species binomial names
  - *Homo sapiens, Pan troglodytes, Canis familiaris, Oryza sativa, Parascaris equorum*
- The number of chromosomes possessed by different species varies and is dependent upon changes that have occurred **during that species' evolution**
- Each individual in a species always has the **same number of chromosomes** (other than in a few rare instances where a chromosome mutation has occurred)
  - **An analogy** is a large, single book containing a trilogy (3) of shorter books; the shorter books could be published separately and still contain the same amount of information
- A **comparison of the chromosome number** of these five selected species can be found below:

Comparison of Chromosome Numbers Table

Name of Species	Diploid Chromosome Number (2n)	Haploid Chromosome Number (n)
Horse threadworm (Parascaris equorum)	4	2
Rice (Oryza sativa)	24	12
Human (Homo sapiens)	46	23
Chimpanzee (Pan troglodytes)	48	24
Dog (Canis familiaris)	78	39

- Note that the diploid number must **always** be an **even number**
  - This is because the diploid number (2n) must always be divisible by two to produce the haploid number (n)
  - The haploid number must always be **a whole number**
- An interesting comparison to make is that the number of chromosomes a species possesses is **not linked** to how 'advanced' a species is in evolutionary terms
  - Chimpanzees and dogs have **more chromosomes than humans** even though they have evolved to be less intelligent and complex than humans

Exam Tip

You may be asked to estimate the number of chromosomes that would be present in the haploid cell of a species. For example, dogs have 78 chromosomes in their diploid cells. When trying to find the number of chromosomes in their haploid cells simply remember that **diploid is 2n** and **haploid is n**, meaning you just need to divide the number of chromosomes by 2. So dogs have 39 chromosomes in their haploid cells!

YOUR NOTES

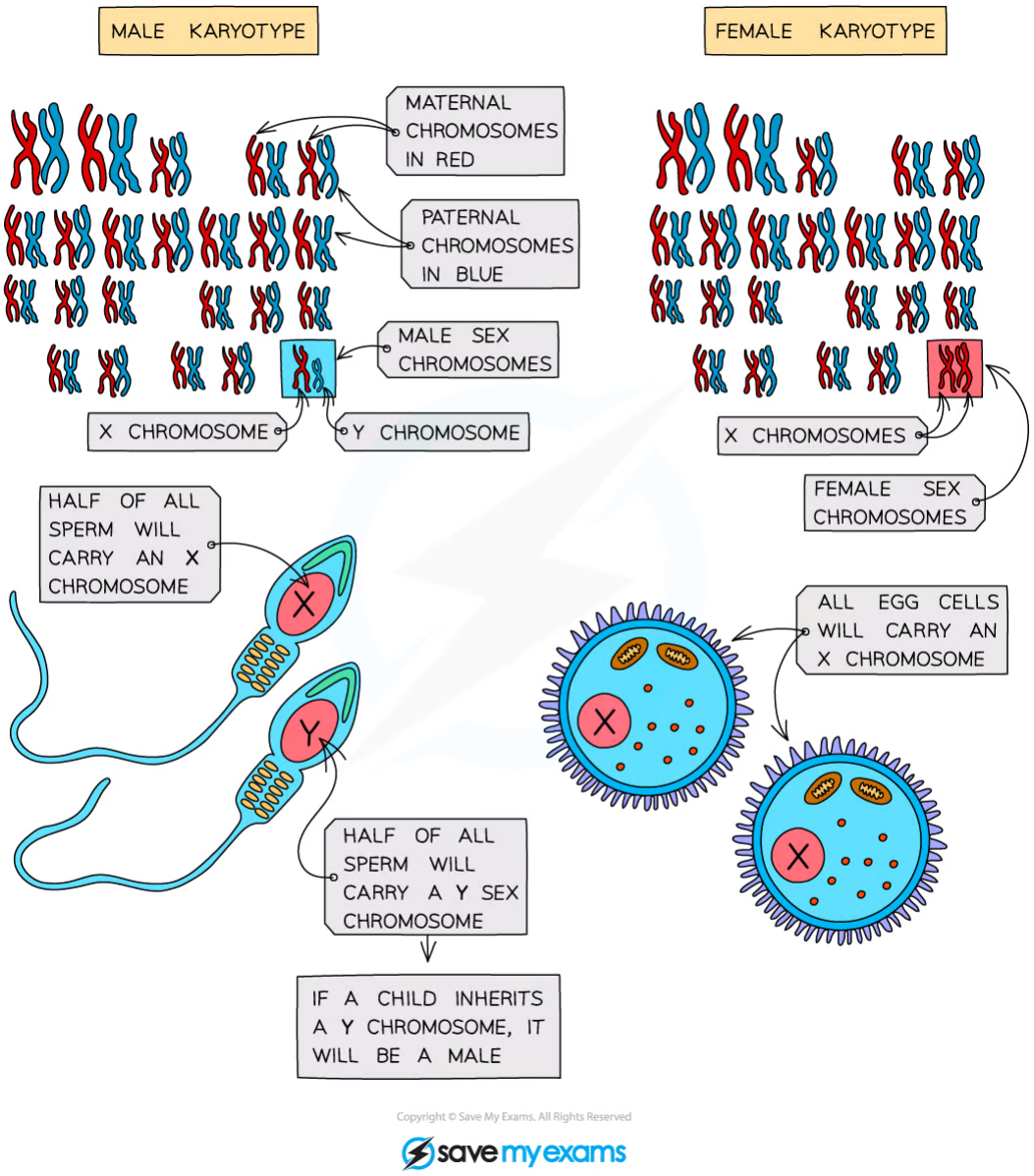
YOUR NOTES

Sex Determination

- Sex is determined by an **entire chromosome pair** (as opposed to most other characteristics that are just determined by one or a number of genes)
- **Females** have the sex chromosomes (pair 23 in humans) **XX**
- **Males** have the sex chromosomes (pair 23 in humans) **XY**
  - Note that the rule XX for females and XY for males applies to mammals, but not to all species

- All other chromosomes (pairs 1 - 22 in humans) are **autosomes** and have **no influence on determining the sex** of offspring
- Because only a father can pass on a Y chromosome, he is **responsible for determining the sex of the child**
  - Due to **meiosis**, half of his sperm cells will carry his X chromosome, half his Y chromosome
  - The chromosome carried by **the sperm that fertilizes the egg** will determine the sex of the child
  - His daughters receive a copy of his X chromosome
  - His sons receive a copy of his Y chromosome

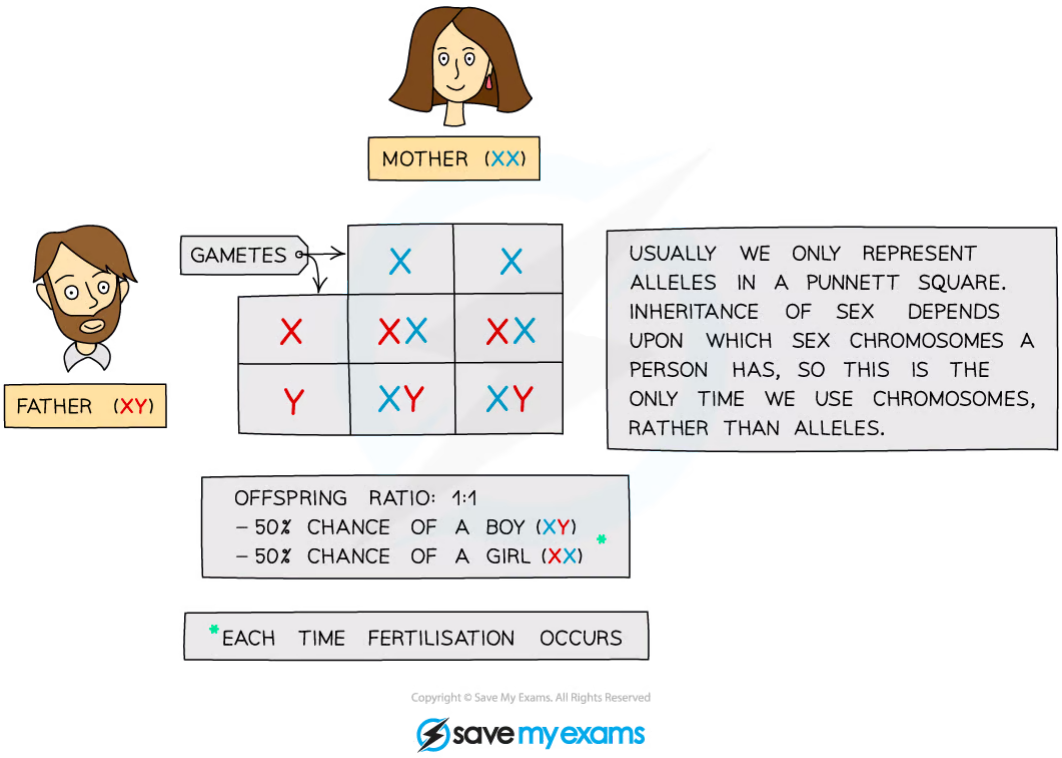
YOUR NOTES



Sperm cells determine the sex of offspring

- The inheritance of sex can be shown using a **genetic diagram** (known as a **Punnett square**), with the X and Y chromosomes taking the place of the alleles usually written in the boxes

YOUR NOTES



Punnett square showing the inheritance of sex

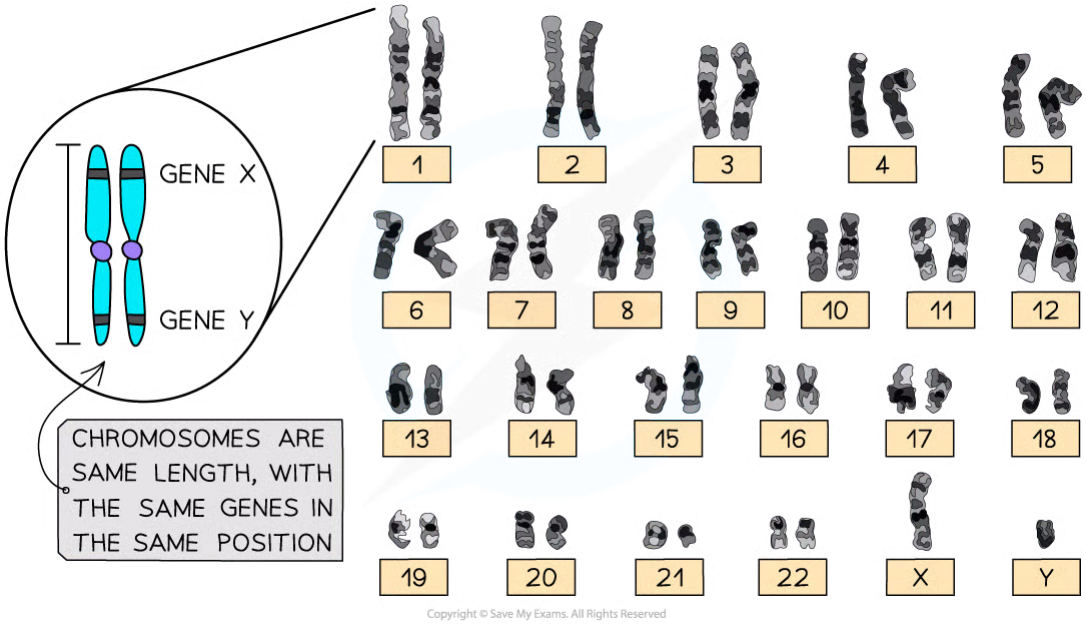
Genes carried by X and Y chromosomes

- The **X chromosome is larger than the Y**, and has its centromere more central than on the Y chromosome
  - Fewer genes are coded for on the Y chromosome as a result
    - The **X carries around 16 × more genes than the Y** chromosome
  - Non-sex phenotypic traits, including **certain blood clotting factors,** are coded for on the X chromosome but not on the Y
- The Y chromosome carries genes that **code for male characteristics**
- One of these genes is the **SRY gene** which is involved in
  - Development of testes in male embryos
  - Production of testosterone
- Females don't receive these genes, so instead, **ovaries develop** and **female sex hormones** are expressed

YOUR NOTES

Karyograms

- During the stages of mitosis, **chromosomes condense** (become visible)
  - This is most notable in **metaphase**
- **Staining** can reveal distinctive banding patterns on chromosomes at this stage
- The **position of the centromere** will also give a clue about which homologous pair a chromosome belongs to
- The process can be frozen in time using **computer image analysis** of all the chromosomes
- Chromosomes can be **placed in their homologous pairs**
- A **karyogram** will show all the chromosomes in homologous pairs, starting with the longest pair and ending with the shortest



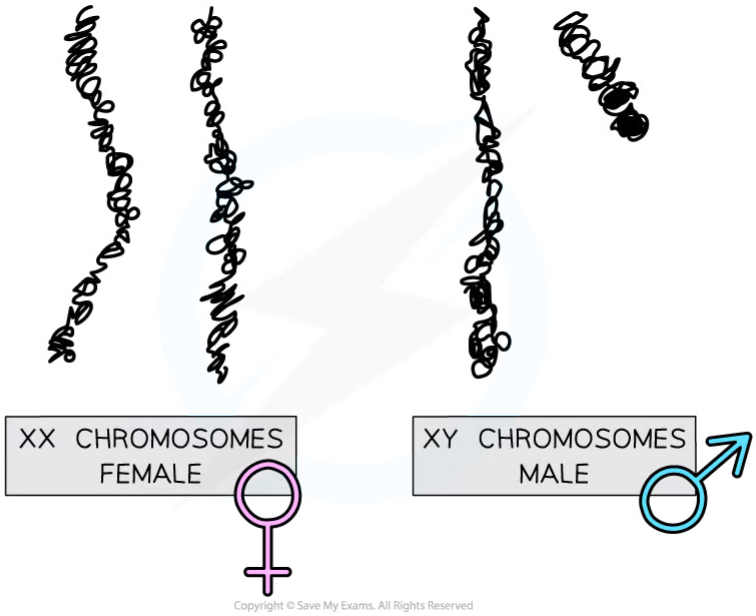
Human karyogram showing homologous chromosomes. The presence of XY reveals this to be a male.

Use of Karyograms

YOUR NOTES

Use of karyotypes to deduce sex in humans

- Karyograms can be used to examine an individual's **karyotype**
- This can reveal the **sex of an individual** by the appearance of the sex chromosome pair
- A Y chromosome is considerably **shorter** than an X chromosome
  - **XX** chromosomes mean an individual is **female**
  - **XY** chromosomes mean an individual is **male**



Appearance of the XX and XY chromosomes. Note the Y chromosome is much shorter than the X.

Use of karyotypes to diagnose Down syndrome in humans

- **Mutations** can occur at different levels, not just mis-copying of individual DNA bases
  - **Chromosome mutations** involve a change in the number of chromosomes
  - A **spontaneous chromosome mutation** called non-disjunction occurs when **chromosomes fail to separate** during meiosis
- The gametes may end up with **one extra copy** of a particular chromosome or no copies of a particular chromosome
- These gametes will have **a different number of chromosomes** compared to the normal haploid number
- Many such gametes will form a non-viable embryo that aborts before becoming a foetus, but not always
- If the abnormal gametes combine in viable fertilization (one that leads to a live birth), then a chromosome mutation occurs as **the diploid cell will have the incorrect number of chromosomes**
- An example of chromosome mutation is **Down syndrome**
- Individuals with Down syndrome have a **total of 47 chromosomes** in their genome as they have three copies of chromosome 21

YOUR NOTES

- Down syndrome is also called **Trisomy 21**

- **Symptoms** include distinctive facial features, hearing loss, learning and growth impairment

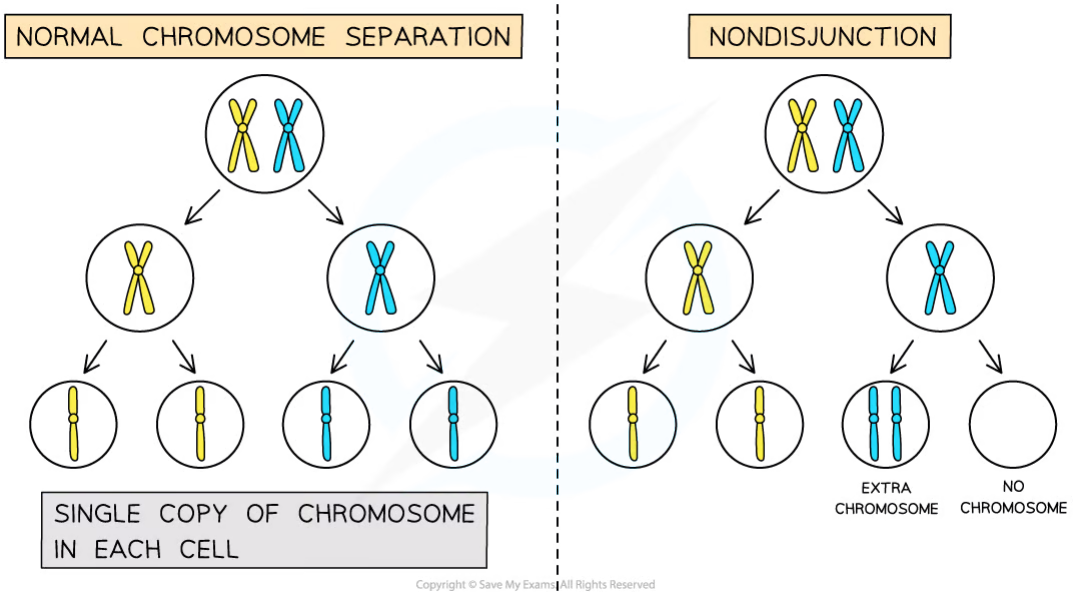


Image showing how chromosomes failing to separate properly during meiosis can result in gametes with the incorrect number of chromosomes

YOUR NOTES

Use of Databases to Identify Gene Loci

Use of databases to identify the locus of a human gene and its polypeptide product

- Following the **sequencing of the whole human genome**, we now know the **exact locus** (position) of every gene across the 23 pairs of chromosomes
- Online databases have been built that are able to **locate any known gene or allele**
- Anyone can access these loci
  - One example is the **European Molecular Biology Laboratory** database (EMBL)
- Examples of genes that can be located are
  - The **CFTR protein**, critical to **cystic fibrosis**, on chromosome 7
  - **HBB**, a faulty allele of which is the cause of **sickle-cell anaemia**, on chromosome 11
- If we know the locus of a particular gene, medicine can establish **the location of a faulty allele**, which is often recessive
  - A faulty allele can be cut out of the chromosome by genetic engineering using **recombinant DNA technology**
  - Replacing a faulty allele could lead to **genetic therapy**
  - Location databases of **cancer-related genes** are often vital information to researchers, doctors and patients involved in cancer genetics

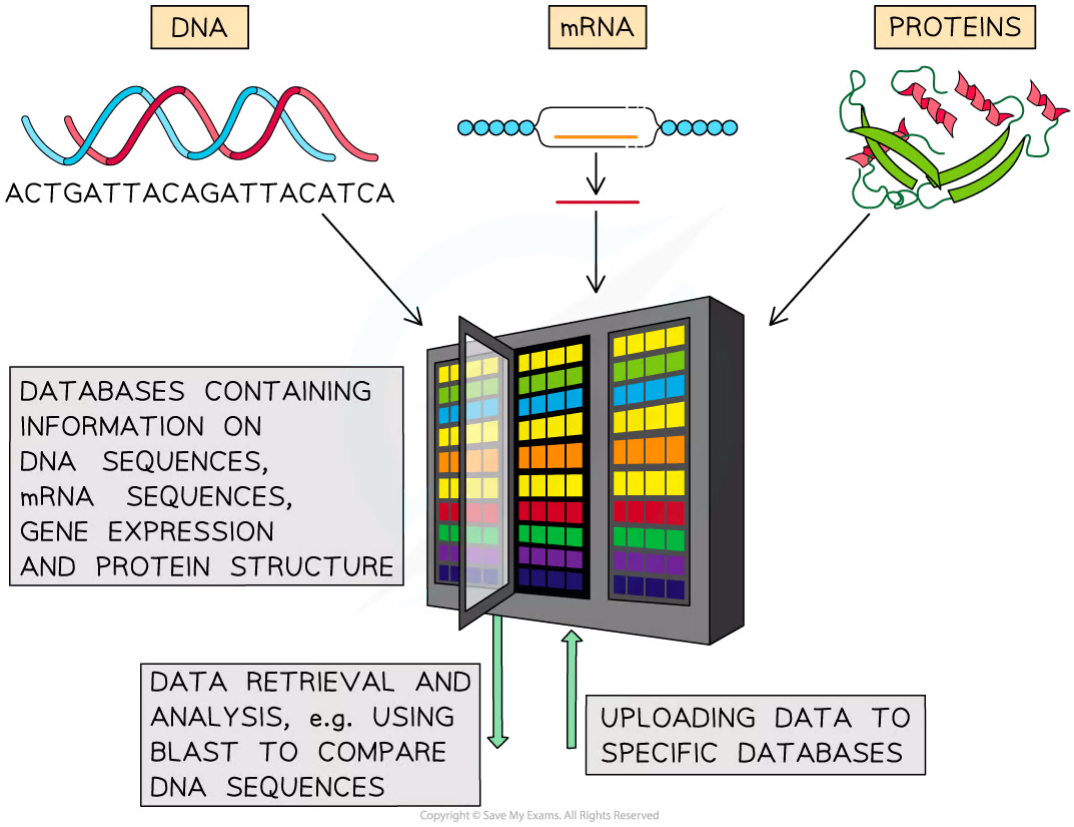
Use of Databases: Comparing Base Sequences

YOUR NOTES

Use of a database to determine differences in the base sequence of a gene in two species

- The Genbank® database is another that can be used to **search for DNA base sequences**
  - Uses a computer data analysis technique called BLAST (Basic Local Alignment Search Tool) to spot and 'line up' similar base sequences

- A protein common to all organisms is **cytochrome C**
- This makes its gene sequence a good one **to compare between organisms**
- The sequence is available for many different organisms **across all three domains**
- This gives important information about **evolutionary relationships** between organisms



The use of databases to compare base sequences (and protein sequences) between species

YOUR NOTES

3.2 Meiosis

3.2.1 Meiosis

Meiosis

- There are two processes by which the **nucleus** of a **eukaryotic cell** can **divide**. These are:
  - Mitosis
  - Meiosis
- **Mitosis** gives rise to **genetically identical cells** and is the type of cell division used for **growth**, **repair** of damaged tissues, **replacement** of cells and **asexual reproduction**
- **Meiosis** gives rise to cells that are **genetically different** from each other and is the type of cell division used to produce **gametes** (sex cells)
- During meiosis, the nucleus of the original 'parent' cell undergoes **two rounds of division**. These are:
  - **Meiosis I**
  - **Meiosis II**

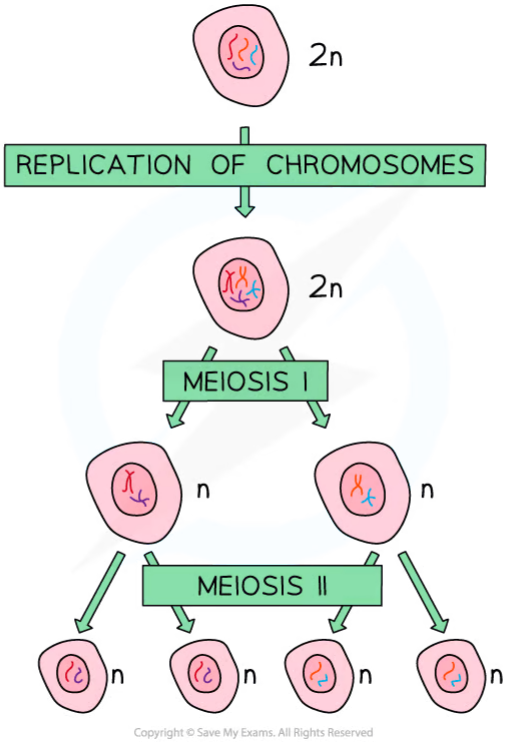
Meiosis I

- The nucleus of the original 'parent' cell is **diploid** (**2n**) i.e. it contains **two sets of chromosomes**
- Before meiosis I, these chromosomes **replicate**
- During meiosis I, the **homologous pairs** of chromosomes are **split up**, to produce **two haploid** (**n**) nuclei
  - At this point, each chromosome still consists of **two chromatids**
- Note that the **chromosome number halves** (from 2n to n) in the **first division** of meiosis (**meiosis I**), not the second division (meiosis II)

Meiosis II

- During meiosis II, the **chromatids** that make up each chromosome **separate** to produce **four haploid** (**n**) nuclei
  - At this point, each chromosome now consists of a **single chromatid**

YOUR NOTES



One diploid nucleus divides by meiosis to produce four haploid nuclei

Discovery of Meiosis

YOUR NOTES

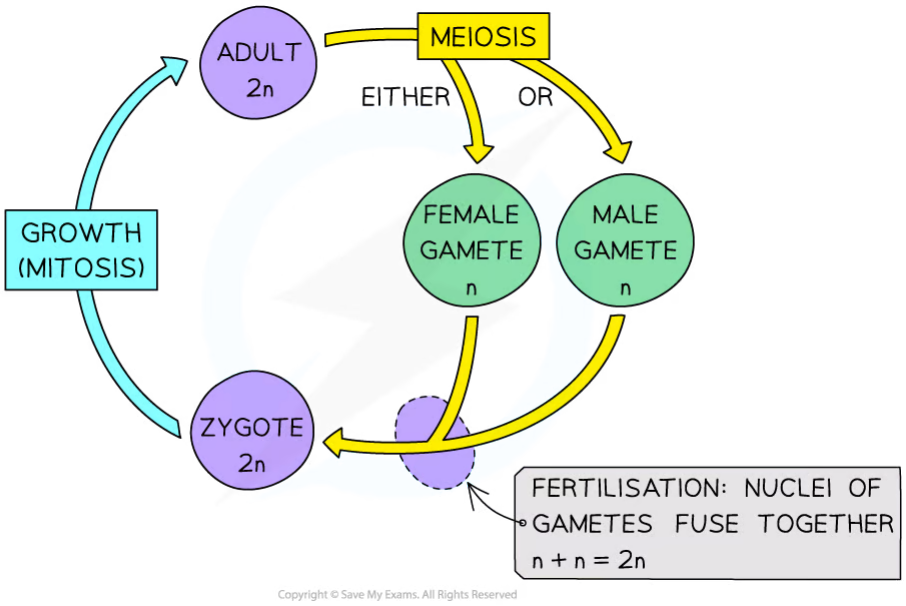
NOS: Making careful observations; meiosis was discovered by microscope examinations of dividing germ-line cells

- In the 19th century, microscopes were developed that could be used to view certain **internal cell structures**
- Around this time, it was also discovered that certain **dyes** could be used to **stain** (and **observe**) the **cell nucleus**
- The use of these dyes eventually led to the discovery of **thread-like structures** inside **dividing nuclei**
  - These were name **chromosomes**
- In the 1880s, a group of German biologists used these new developments to make detailed observations of dividing nuclei
  - Their careful observations led to **the discovery of the process of meiosis** and a basic understanding of how it occurs
- One key observation was made by viewing the chromosomes in specific cells in an organism known as the horse threadworm (*Parascaris equorum*)
  - It was observed that the **nuclei** of their **egg** and **sperm** cells contained **two chromosomes**, whereas the nuclei of a **fertilised egg** contained **four chromosomes**
  - This suggested that the chromosome number had been doubled by the process of fertilisation
  - This led to the **hypothesis** that, at some point in every generation, a **special type of nuclear division** must occur that **halves the chromosome number**
- The **specific sequence of events in meiosis** was finally discovered by carefully observing cells from the **ovaries** of **European rabbits** (*Oryctolagus cuniculus*) between 0 and 28 days old
  - This was possible because in females of this species, certain cells in the ovaries start undergoing meiosis **from birth** and the process continues **slowly** over a period of **many days**
- The initial discovery of meiosis (as well as the following series of discoveries that revealed to scientists how it occurs) was made possible through **careful scientific observations**
- Such careful observations are needed in order to **validate** the **claims** and **discoveries** that scientists make, as **scientific evidence** is required. Careful observations can enable scientists to collect **evidence that allows theories to be developed**
- The use of apparatus, dyes, sensors, amongst many other scientific tools, allows scientists to make these **precise and accurate observations**
- The methods used to make such observations must be able to be **repeated** so that the results can be confirmed by other scientists if necessary

YOUR NOTES

Sexual Life Cycle

- The life cycles of organisms can be **sexual** or **asexual** (some organisms are capable of both)
  - In an **asexual** life cycle, the **offspring are genetically identical to the parent** (they have exactly the same chromosomes)
  - In a **sexual** life cycle, the **offspring are genetically distinct from each other and from each of the parents** (their chromosomes are different, causing them to be genetically distinct)
- The **halving of the chromosome number** during meiosis is very important for a **sexual life cycle** as it allows for the **fusion of gametes**
- Sexual reproduction is a process involving the **fusion** of the nuclei of two gametes to form a **zygote** (fertilised egg cell) and the production of offspring that are genetically distinct from each other
- This **fusion of gamete nuclei** is known as **fertilisation**
  - Fertilisation **doubles** the number of chromosomes each time it occurs
  - This is why it is essential that the chromosome number is also **halved** at some stage in organisms with a **sexual life cycle**, otherwise the chromosome number would keep doubling every generation
  - This halving of the chromosome number occurs during **meiosis**
  - In animals, this halving occurs during the **creation of gametes**

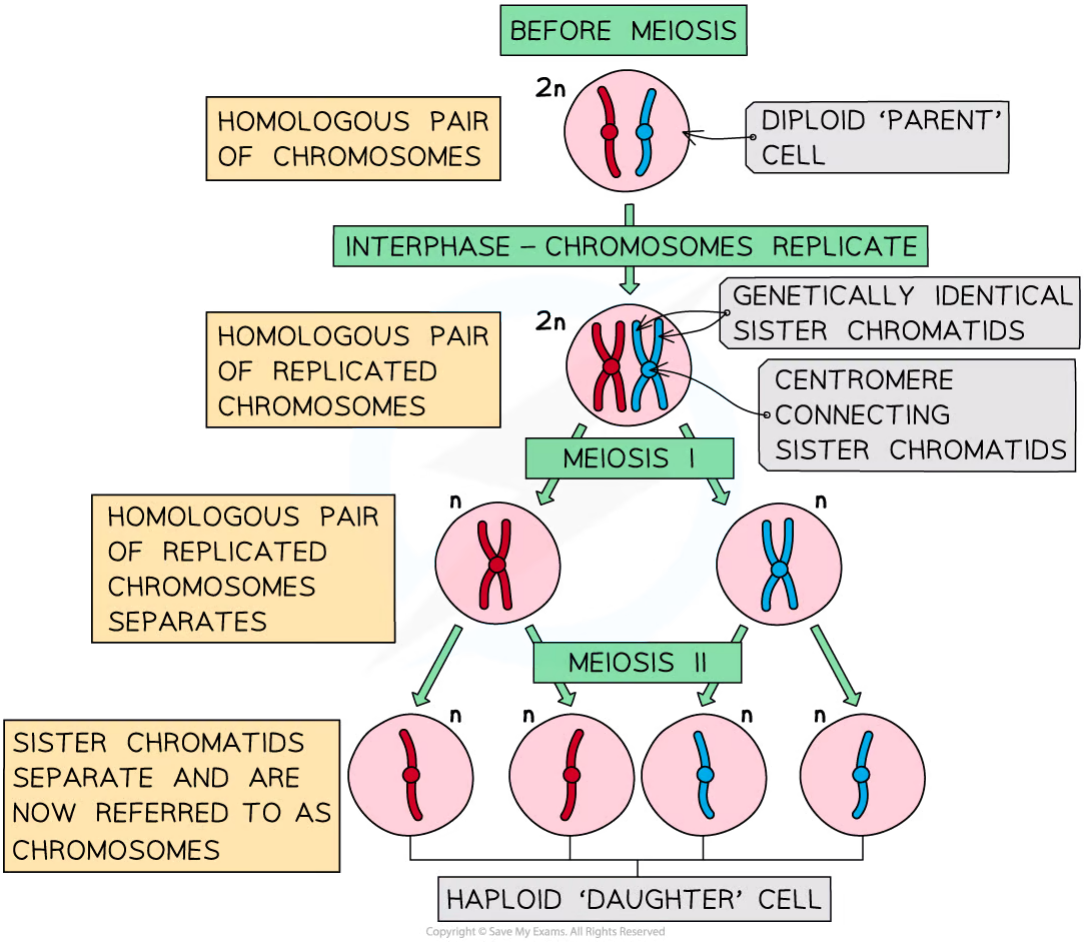


Sexual life cycle

YOUR NOTES

DNA Replication before Meiosis

- **Before meiosis occurs**, all of the DNA inside the nucleus of the 'parent' cell is **replicated**
  - This occurs during a period of the cell cycle known as **interphase**
- Once this has occurred, each chromosome now consists of **two genetically identical sister chromatids**, which are joined together by a **centromere**
  - The sister chromatids are genetically identical because DNA replication is a very accurate process and only a very small number of mistakes occur when DNA is being copied
- The two DNA molecules formed by DNA replication prior to meiosis are considered to be **sister chromatids** until the **splitting of the centromere** at the start of **anaphase** (a stage during meiosis II, during which the sister chromatids are **pulled apart**)
- After this, they are once again considered as **individual chromosomes**



DNA replication before meiosis

Exam Tip

Understanding the difference between chromosomes and chromatids can be difficult. We count chromosomes by the **number of centromeres present**. So when the 46 chromosomes duplicate during interphase and the **amount of DNA in the cell doubles** there are still only 46 chromosomes present because there are still only 46 centromeres present. However, there are now 92 chromatids, which are strands of replicated chromosomes.

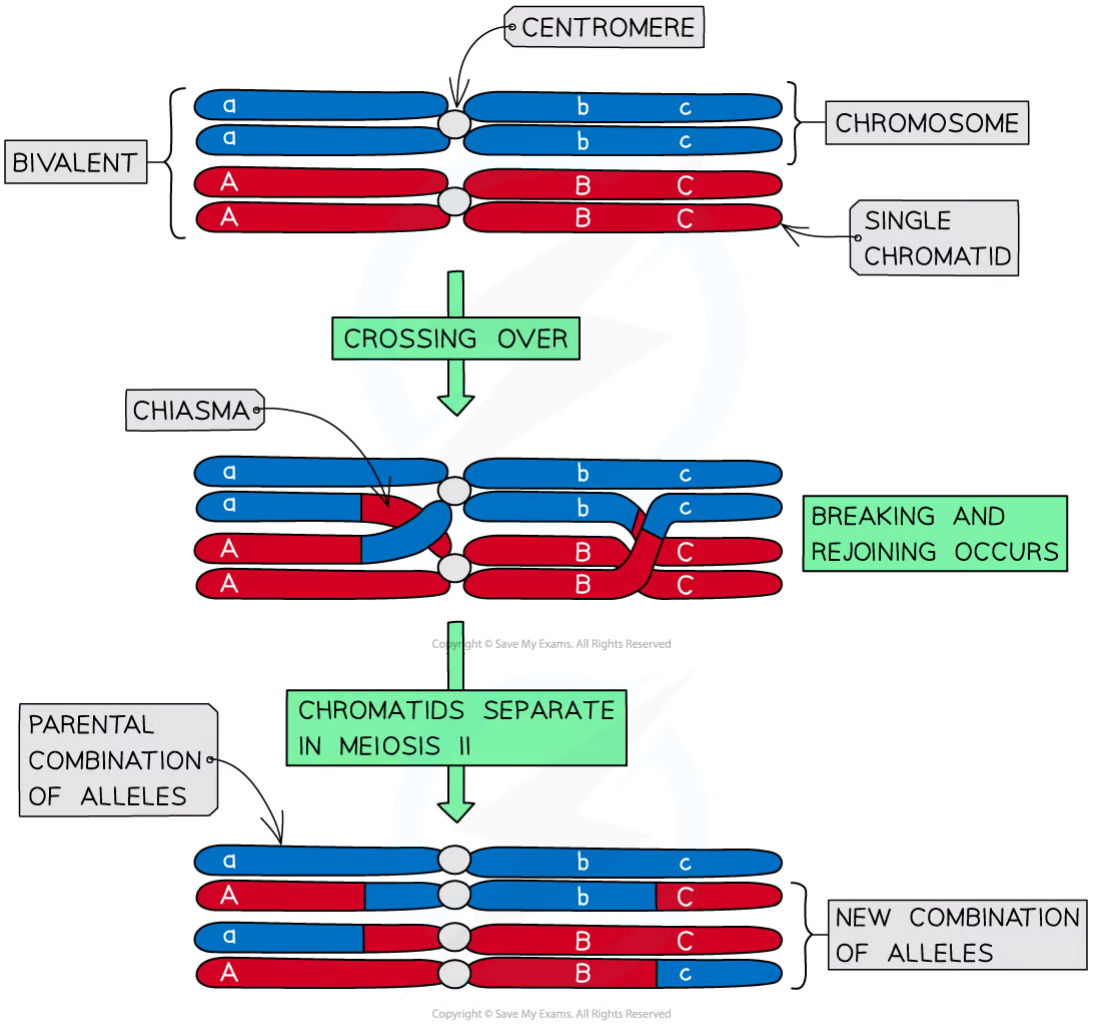
YOUR NOTES

Formation of Bivalents & Crossing Over

YOUR NOTES

- At the start of meiosis, **homologous chromosomes pair up** with each other
  - As DNA replication has already occurred, each chromosome is made up of **two sister chromatids**
  - This means that a pair of homologous chromosomes is made up of **four DNA molecules**
- A pair of homologous chromosomes is known as a **bivalent**
- The pairing process resulting in the formation of a bivalent is known as **synapsis**
- After synapsis has occurred, a process known as **crossing over** may occur
- During crossing over, two **non-sister chromatids** (i.e. one chromatid from each of the homologous chromosomes) form a **junction**
- At this junction, the two chromatids **break** and **rejoin** with each other
- As these crossover events occur at exactly the same position on the two non-sister chromatids, this allows **genes** to **exchange** between the chromatids
- Non-sister chromatids are **homologous** but are **not genetically identical** and this means that some of the **alleles** of the exchanged genes will be **different**
- This process, therefore, produces chromatids with **completely new combinations of alleles** (that were not previously present in the DNA of the 'parent' cell)
- As these chromatids will eventually be split up into **different gametes**, crossing over is of great **importance** because it is a significant source of **genetic variation** between gametes
  - This ensures there is genetic variation in **populations** of **sexually-reproducing species**, which is key to a species' ability to **evolve** and **adapt** to changes in its environment over time

YOUR NOTES

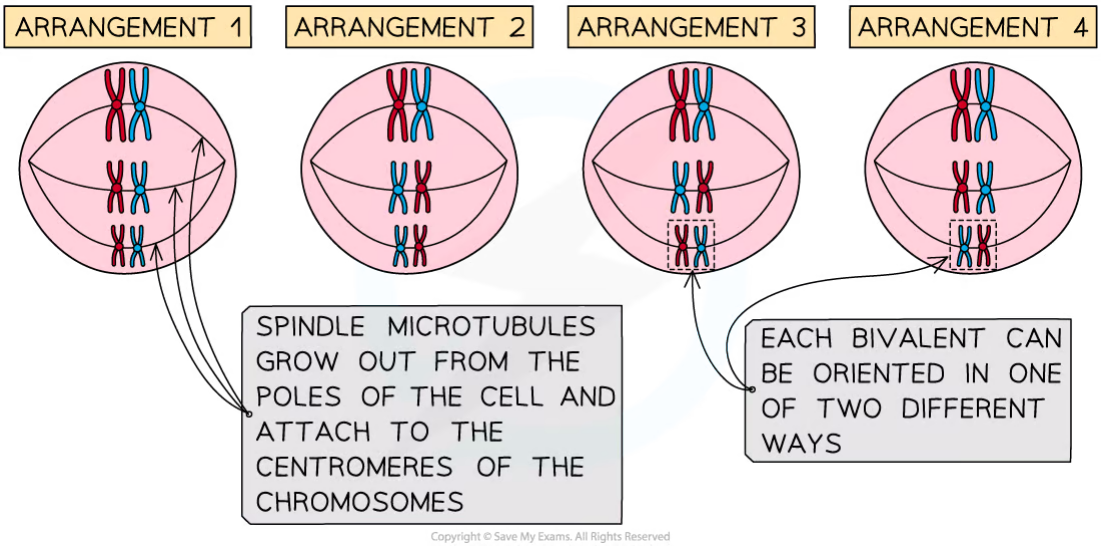


Crossing over of non-sister chromatids leading to the exchange of genetic material

YOUR NOTES

Random Orientation

- At metaphase, during meiosis I, **homologous chromosomes line up at the cell equator** as they prepare to **separate**
- **Spindle microtubules** grow out from the **poles** of the cell and attach to the **centromeres** of the chromosomes
- Each of the two homologous chromosomes in a bivalent is attached to a **different pole**
- The **orientation of the bivalents** when they line up at the cell equator determines which pole each chromosome gets attached to (and eventually pulled towards)
- The orientation of the bivalents is **completely random**
- In addition, the bivalents also **assort independently of one another** (i.e. the orientation of one bivalent never affects the orientation of another)



The orientation of bivalents lining up at the cell equator is random

Reduction Division

- During meiosis, the homologous chromosomes forming a bivalent separate in a process known as **disjunction**
- The homologous chromosomes then move to **opposite poles** of the cell
- As **one chromosome of each type** moves to each pole, the two separate nuclei formed by the first division of meiosis (meiosis I) now only contain **one of each type of chromosome**, making the two new cells **haploid**
  - Essentially, the chromosome number of the cells has been **halved**
- This is why the first division of meiosis is known as a **reduction division**
  - The chromosome number has been **reduced** (halved) from **diploid** to **haploid**

YOUR NOTES

Genetic Variation & Meiosis

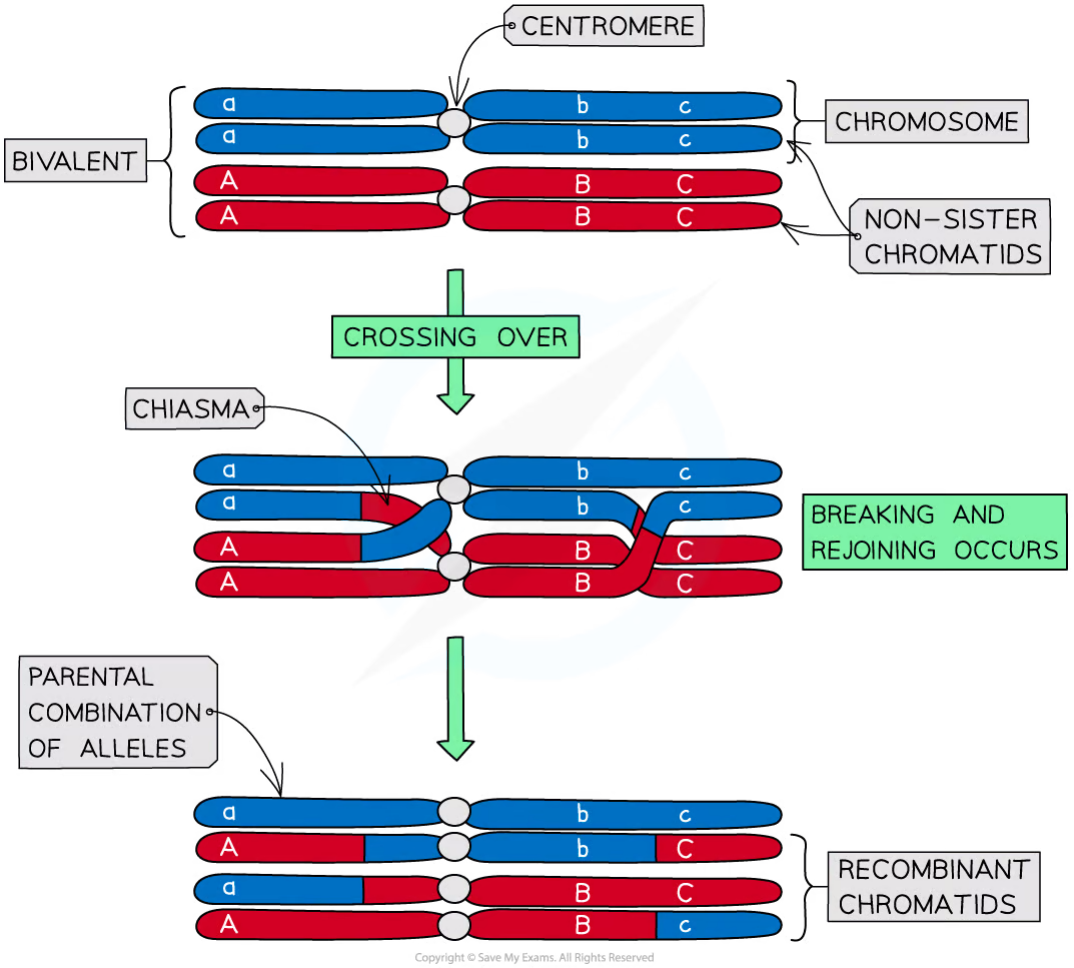
Crossing over and random orientation promote genetic variation

- Having **genetically different offspring** can be **advantageous** for natural selection and therefore increase the survival chances of a species
- **Meiosis** has several mechanisms that **increase the genetic variation of gametes** produced
- Both **crossing over** and **random orientation** result in **different combinations of alleles** in gametes

Crossing over

- Crossing over is the process by which **non-sister chromatids exchange alleles**
- Process:
  - During prophase I of meiosis homologous chromosomes pair up and are in very close proximity to each other
    - A pair of homologous chromosomes can be referred to as a **bivalent**
  - At this point, there can be an exchange of genetic material **(alleles)** between **non-sister chromatids** in the bivalent
  - The crossing points are called **chiasmata**
  - This results in a **new combination of alleles on the two chromosomes** (these can be referred to as **recombinant chromosomes**)
- This swapping of alleles is a significant source of genetic variation because it can occur at multiple random positions along the chromosome
- Crossing over can happen anywhere along the chromosome but is more likely to occur further down the chromosome away from the centromere

YOUR NOTES

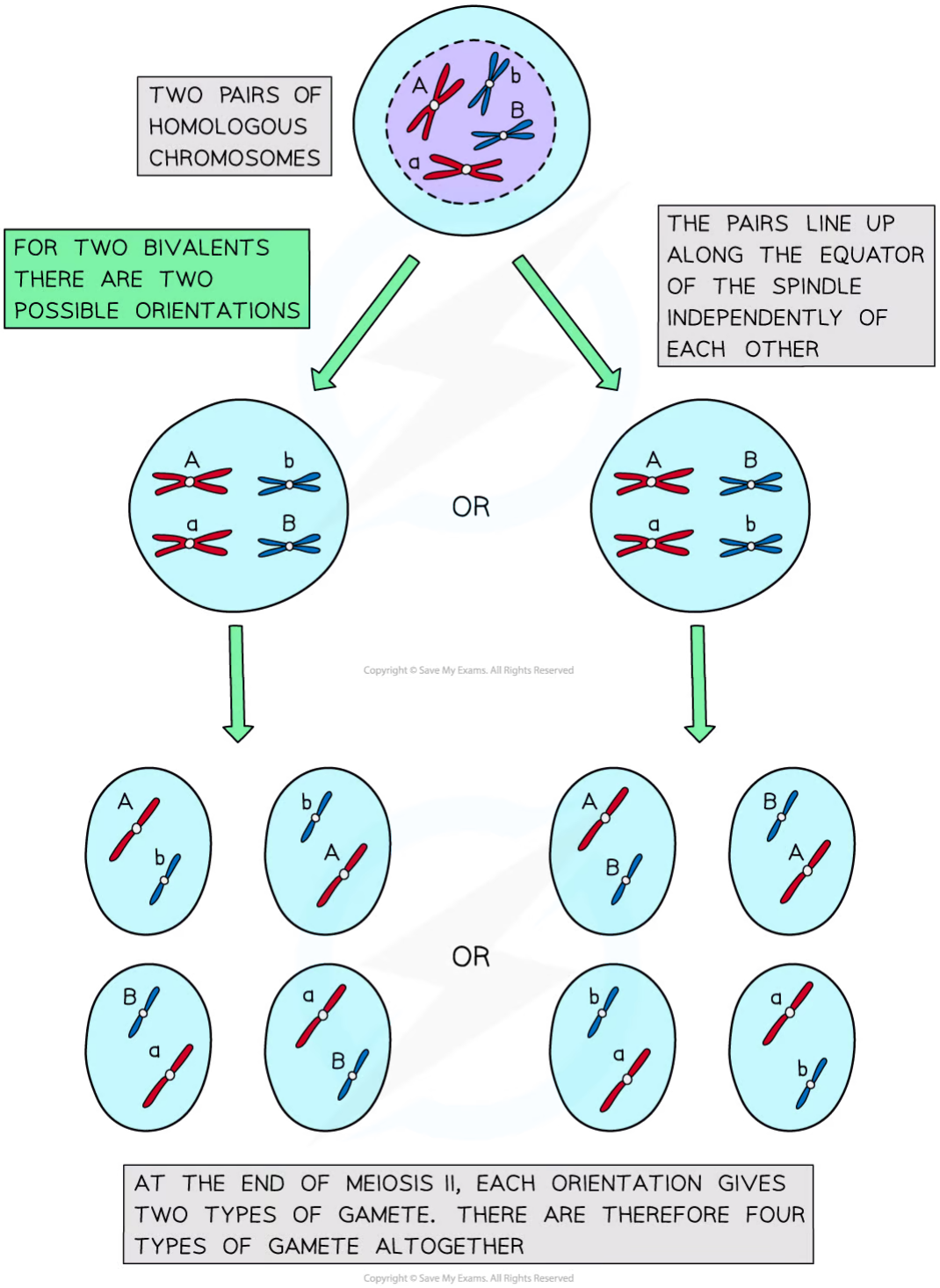


Crossing over occurring between two non-sister chromatids

Random orientation

- The **random orientation of homologous pairs along the equator of the cell** during **metaphase I** result in the production of **different allele combinations in daughter cells**
- In prophase I, homologous chromosomes pair up and in metaphase I, they align along the equator of the cell
  - Each pair can be arranged with **either chromosome on top**, this is completely random
  - The **orientation of each homologous pair** is **random/independent** (unaffected by the orientation of any other pair)
- In anaphase I the homologous chromosomes are **separated** and pulled apart to different poles
- The combination of alleles that end up in each daughter cell depends on how the pairs of homologous chromosomes were lined up
  - The different combinations of chromosomes in daughter cells increases genetic variation between gametes

YOUR NOTES



The random orientation of homologous chromosomes leads to different genetic combinations in daughter cells

The different combinations of chromosomes following meiosis

- The number of possible chromosomal combinations resulting from random assortment is equal to 2^n

YOUR NOTES

- *n* is the number of homologous chromosome pairs or haploid number

- For humans: the number of chromosomes is 46 meaning the number of homologous chromosome pairs is 23 so the calculation would be:
  - 2^{23} = 8,388,608 possible chromosomal combinations

Worked Example

Calculate how many different chromosomal combinations can result from meiosis in a plant species which has a diploid number of 16. Assume no crossing over occurs.

[1 mark]

Step 1: Use the relevant formula

$$2^n$$

Step 2: Calculate the haploid number

$$\text{Diploid number (2n)} = 16$$

$$\text{Haploid number (n)} = 16 \div 2 = 8$$

Step 3: Substitute in figures

$$2^8 = 256$$

There are **256** different chromosomal combinations that can occur.

Genetic Variation & Fertilisation

YOUR NOTES

Fusion of gametes from different parents promotes genetic variation

- **Meiosis** creates genetic variation **between the gametes** produced by an individual through crossing over and random orientation
- This means each gamete carries substantially **different alleles**
- During fertilisation, any male gamete can fuse with any female gamete to form a zygote
- This **random fusion of gametes** at fertilisation creates genetic variation **between zygotes** as each will have a unique combination of alleles

The different combinations of chromosomes following fertilisation

- In random fertilisation, **any two gametes may fuse together**
- Therefore the formula to calculate the number of combinations of chromosomes after the random fertilisation of two gametes is $\mathbf{(2^n)^2}$
  - *n* is the haploid number and ^{2} is the number of gametes
  - Therefore in humans, when the haploid number is 23, the number of combinations following fertilization is $(2^{23})^2 = 70{,}368{,}744{,}177{,}664$
- This explains why relatives can differ so much from each other. Even with the same parents, individuals can be genetically distinct due to variation at the meiosis and fertilization stage (as well as other possible mutations and crossing-over)

Worked Example

Calculate the number of different possible chromosome combinations after the random fertilization of an ovule and pollen nuclei from the same plant species (Diploid number = 16).

[2 marks]

Step 1: State formula for random fertilisation between any two gametes

$$(2^n)^2$$

Step 2: Use information from question to state haploid number

$$n = 8$$

Step 3: Substitute in figures

$$(2^n)^2$$

$$(2^8)^2$$

Answer **65,536**

Exam Tip

These sources of genetic variation explain why relatives can differ so much from each other. Even with the same parents, individuals can be genetically distinct due to the processes outlined above.While we can calculate the number of chromosomal combinations that result from random orientation and random fertilisation, the number of combinations from crossing over is infinite, or as good as!

YOUR NOTES

YOUR NOTES

Non-disjunction

- **Non-disjunction** occurs when chromosomes fail to separate correctly during **meiosis**
- This can occur in either anaphase I or anaphase II, leading to **gametes** forming with an **abnormal number of chromosomes**
  - The gametes may end up with **one extra copy** of a particular chromosome or **no copies** of a particular chromosome
  - These gametes will have a different number of chromosomes compared to the normal haploid number
- If the abnormal gametes are fertilized, then a **chromosome abnormality** occurs as the diploid cell (**zygote**) will have the incorrect number of chromosomes

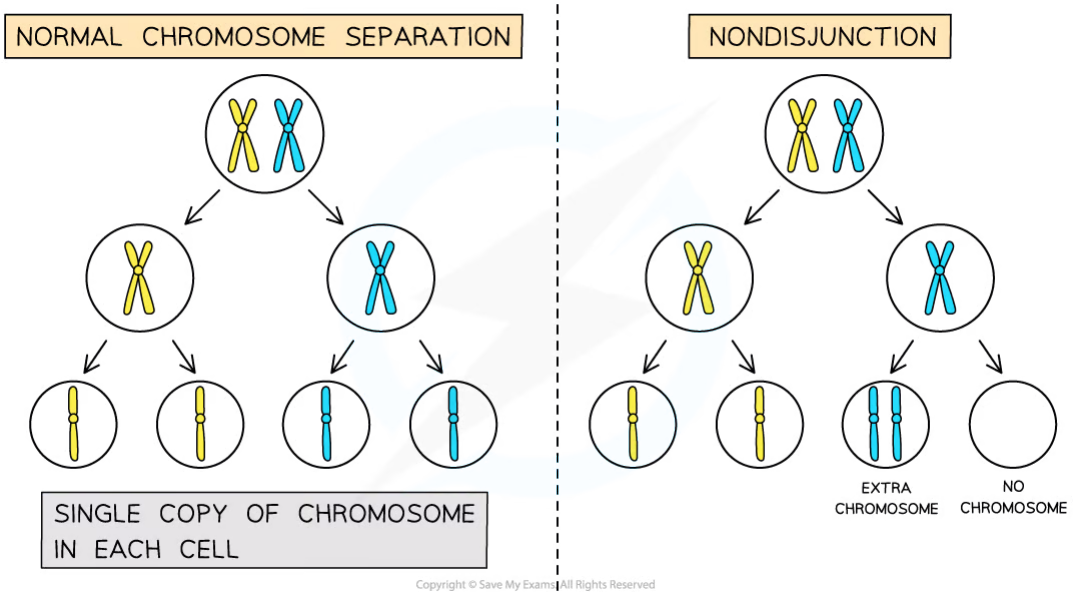


Image showing how chromosomes failing to separate properly during meiosis can result in gametes with the incorrect number of chromosomes

Down Syndrome

- A key example of a non-disjunction chromosome abnormality is Down syndrome, also called **Trisomy 21**
- Non-disjunction occurs during anaphase I (in this case) and the 21st pair of homologous chromosomes fail to separate
- Individuals with this syndrome have a total of 47 chromosomes in their cells as they have three copies of **chromosome 21**
- The impact of trisomy 21 can vary between individuals, but some common features of the syndrome are **physical growth delays** and **reduced intellectual ability.** Individuals can also suffer from issues with **sight** or **hearing**

Other trisomy syndromes

YOUR NOTES

- There are other trisomy possibilities that can result from non-disjunction; many, but not all, have very serious impacts on the phenotype of the offspring which may be fatal
  - **Patau syndrome** (trisomy 13) and **Edwards syndrome** (trisomy 18) are very serious syndromes which result in many physical disabilities and developmental difficulties
    - Trisomy 18 and 13 both have **very low survival rates** with few babies surviving past their first birthday
  - **Klinefelter's syndrome** is caused by non-disjunction in sex chromosomes which leads to having the chromosomes XXY
    - This syndrome is often not diagnosed until adulthood and doesn't impact life expectancy but may have a negative effect on fertility
  - **Turners syndrome** also affects the sex chromosomes with individuals possessing just one X chromosome
    - Individuals with Turners syndrome would not necessarily have a reduced life-expectancy, although will often be shorter and may suffer some symptoms such as lack of sexual development during puberty

Age & Non-disjunction

- Many studies have shown that there is a correlation between age and the incidence of non-disjunction
- It is believed that as the **age of the parents increases** the **incidence of non-disjunction increases**
- In particular, the age of the **mother** has been found to increase the chance of having a child with Down Syndrome
  - The impact of age on the risks is represented in the table below

Age and Risk of Down's Syndrome Table

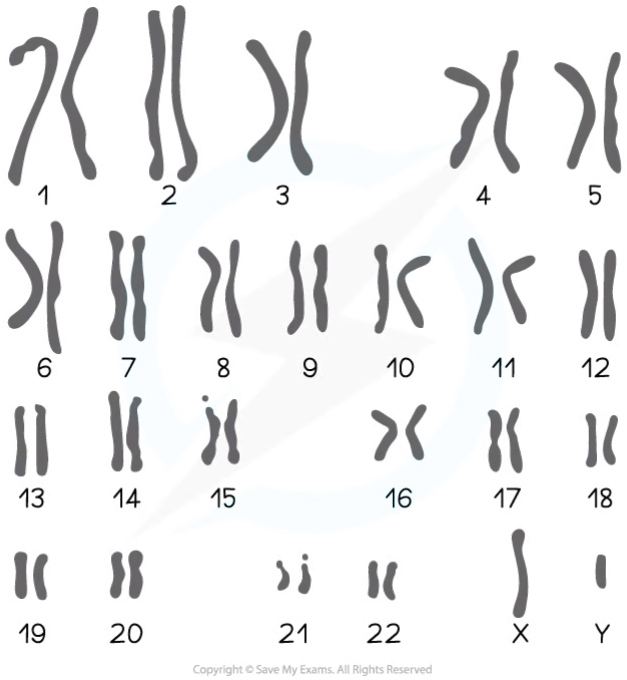
Mother's age (Years)	25–29	30–34	35–39	40–44	45+
Chance of having baby with Down Syndrome	1 in 1250 live births	1 in 1000 live births	1 in 400 live births	1 in 100 live births	1 in 30 live births

YOUR NOTES

Methods used for Karyotype Analysis

Methods can be used to obtain cells for karyotype analysis

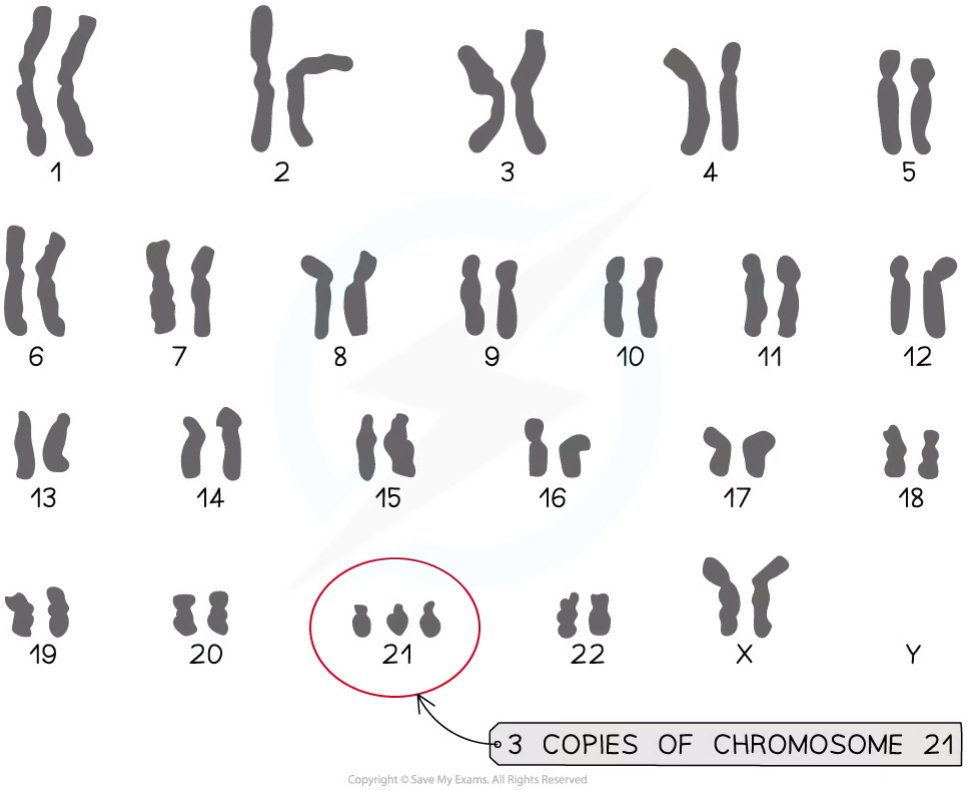
- A **karyotype** can be created to show an image of all of the chromosomes of an individual from a single cell.
- Chromosomes are arranged into their homologous pairs and studied to check for any abnormalities



This karyogram shows a typical or "normal" karyotype

- A karyotype which shows a chromosomal abnormality may have incorrect numbers of chromosomes present
- Trisomy syndromes will show a third chromosome present at one of the chromosome positions
  - For example, Down's syndrome shows a third chromosomes at the 21st position

YOUR NOTES



A karyogram showing the karyotype of an individual with Down's Syndrome

- Two methods can be used to obtain cells from an **unborn child** for chromosome testing:
  - **Amniocentesis**
  - **Chorionic villus sampling**

Amniocentesis

- A needle is inserted through the mother's abdomen wall and a small sample of **amniotic fluid** is taken. The sample will contain some foetal cells for analysis
- This procedure usually takes place around 16 weeks of pregnancy
- There is a **small risk of miscarriage** associated with the procedure (approx 1%)
- The procedure also poses a small risk of infection

Chorionic Villus Sampling (CVS)

- A long tube is inserted through the vagina and then cervix in order to take a small sample of the developing **chorion** (a membrane surrounding the embryo which forms part of the placenta)
- This procedure can be carried out earlier in the pregnancy; around 10 - 12 weeks
- CVS has a slightly **increased risk of miscarriage** associated with the procedure (approx 2%)
- There is also a small risk of infection

YOUR NOTES

Drawing the Stages of Meiosis

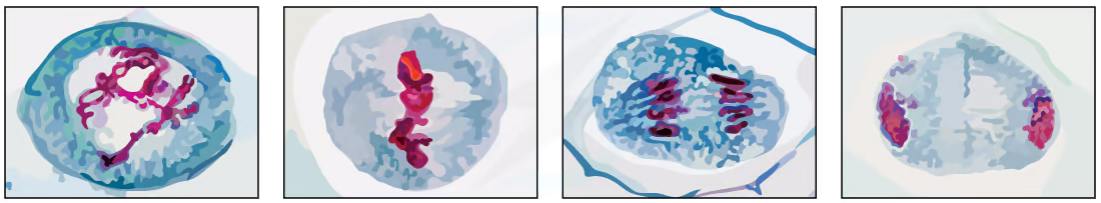
- Cells undergoing meiosis can be observed and photographed using specialized microscopes
- The different stages of meiosis have distinctive characteristics meaning they can be identified from photomicrographs
- Being able to identify the stages of meiosis from photomicrographs and diagrams is an important skill for a biologist

Step 1: Identifying if meiosis I or meiosis II is occurring

- **Homologous chromosomes** pair up side by side in meiosis I only
  - This means if there are **pairs of chromosomes** in a diagram or photomicrograph **meiosis I** must be occurring
- The **number of cells forming** can also help identify whether meiosis I or II is occurring
  - If there are **two new cells** forming it is **meiosis I** but if there are **four new cells** forming it is **meiosis II**

Identifying which stage of meiosis I is occurring

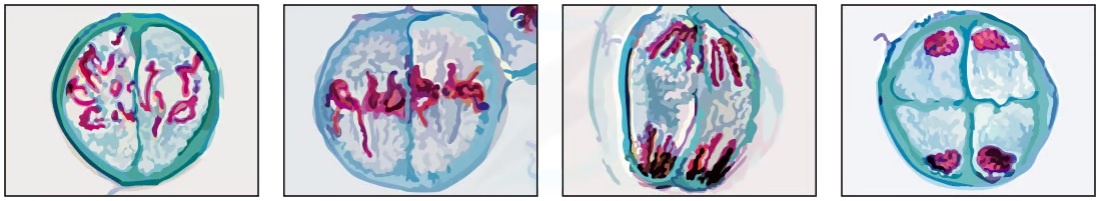
- **Prophase I**: **Homologous pairs** of chromosomes are visible in diploid cell (2n). Crossing over occurs
- **Metaphase I**: Spindle fibres pull homologous pairs so they are lined up **side by side** along the **equator** of the cell. Orientation of homologous chromosomes is random
- **Anaphase I**: **Whole chromosomes** are being pulled to opposite **poles** with **centromeres intact**
- **Telophase I:** There are **2 groups** of condensed chromosomes around which nuclei membranes are forming
- **Cytokinesis:** Cytoplasm is dividing and the **cell membrane is pinching inwards** to form **two cells** with haploid chromosome numbers (n)



Prophase I, Metaphase I , Anaphase I and Telophase I as seen in photomicrographs

Identifying which stage of meiosis II is occurring

- **Prophase II**: **Single whole chromosomes** are visible in haploid cells
- **Metaphase II**: Single whole chromosomes are lined up along the **equator** of the cell in a **single file**
- **Anaphase II**: **Centromeres divide** and **chromatids** are being pulled to opposite **poles**
- **Telophase II:** Nuclei are forming around the **4 groups** of condensed chromosomes
- **Cytokinesis**: Cytoplasm is dividing and **four haploid cells** are forming



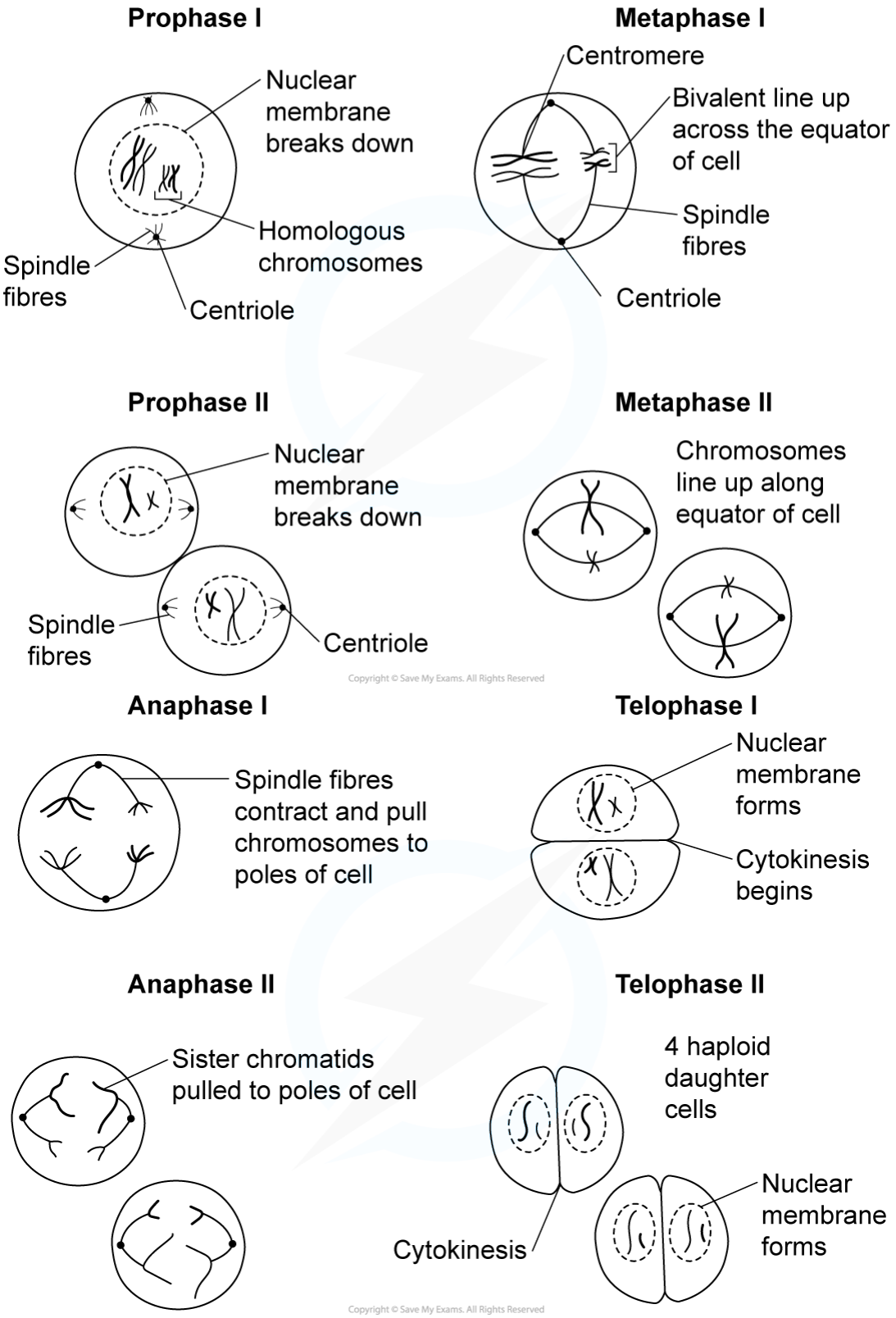
Prophase II, Metaphase II , Anaphase II and Telophase II as seen in photomicrographs

Drawing the stages of meiosis

- The distinguishing features mentioned above can also be used by biologists to draw scientific diagrams of meiosis I and meiosis II
- The conventions for drawing are:
  - The drawing must have a title
  - A **sharp HB pencil** should be used (and a good eraser!)
  - Drawings should be on plain white paper
  - Lines should be **clear**, **single lines** (no thick shading)
  - **No shading**
  - The drawing should take up as much of the space on the page as possible
  - Well-defined structures should be drawn
  - The drawing should be made with **proper proportions**
  - **Label lines** should not cross or have arrowheads and should **connect directly** to the part of the drawing being labelled

YOUR NOTES

YOUR NOTES



Drawing the different stages or phases of meiosis

Exam Tip

For metaphase remember **M for the middle** of the cell which is where the chromosomes will be lined up.For anaphase remember **A for away** from the middle to the poles, which is where the chromosomes / chromatids are being pulled.When drawing the stages of meiosis you do not have to show crossing over occurring.

YOUR NOTES

YOUR NOTES

3.3 Inheritance

3.3.1 Inheritance

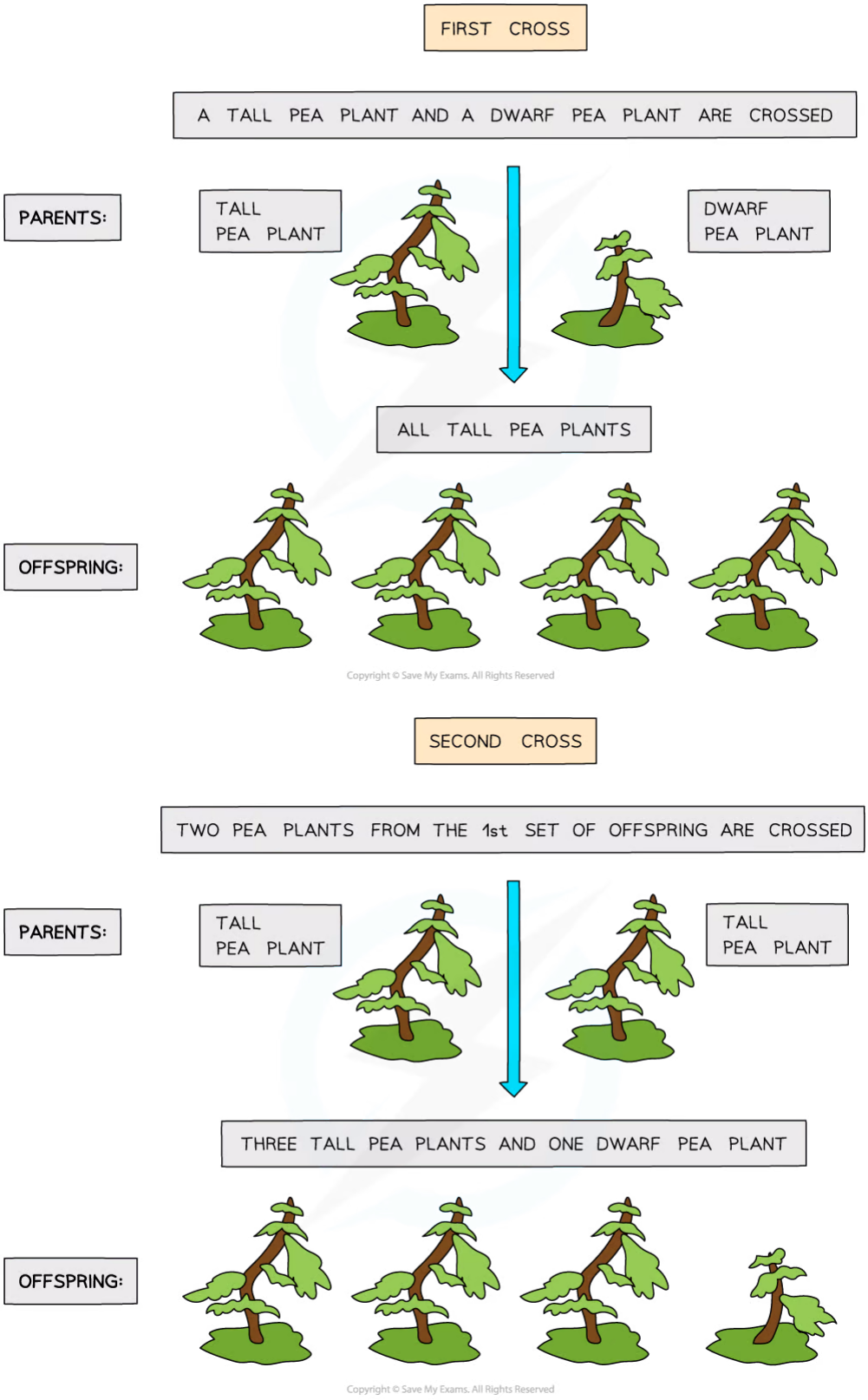
Mendel's Experiments

- **Gregor Mendel** was an Austrian monk
- He was trained in mathematics and natural history at the University of Vienna
- In the mid-19th century, Mendel carried out breeding experiments on large numbers of pea plants whilst looking after the monastery gardens
- He studied how **characteristics were passed on between generations of plants**
- Due to his extensive work on the understanding of inheritance, he is sometimes called the **father of genetics**

Mendel's groundbreaking work

- Mendel carefully **transferred pollen** from one pea plant to the reproductive parts of another
- This technique **eliminated any uncertainty** from his data since he knew which plants were fertilized by which pollen
- He **collected the pea seeds from these plants** and grew them in favourable conditions to find out their characteristics
- He also **cross-bred offspring peas** in order to find out which, if any characteristics would appear in future generations
- Mendel investigated the **height** of pea plants, the **colours of their flowers** and the **smoothness of their seed coat**

YOUR NOTES



Mendel's pea plant crosses

YOUR NOTES

Mendel's Pea Plant Results Table

Parental characteristics	Characteristics of first generation plants	Characteristics of second generation plants	Ratio of characteristics in second generation
Tall plant × dwarf plant	100% tall plants	868 tall plants and 277 dwarf plants	3.1 : 1
Round seed coat × wrinkled seed coat	100% Round seed coat	5474 round seed coat and 1850 wrinkled seed coat	3 : 1
Purple flowers × white flowers	100% Purple flowers	705 purple flowers and 224 white flowers	3.1 : 1

- Mendel found that characteristics were **inherited** in a **predictable pattern**
- **All** pea plants in the first generation had the same characteristic as **one** of the parental plants
- The offspring plants in the second generation had characteristics of **both** parent plants in a **3:1 ratio**
- Without knowing it, Mendel had discovered genes, he referred to them as **'units of inheritance'**
- He also discovered that some genes are **dominant** and some genes are **recessive**
- Different forms of the same gene are called **alleles**

Mendel's Experimental Technique

NOS: Making quantitative measurements with replicates to ensure reliability; Mendel's genetic crosses with pea plants generated numerical data

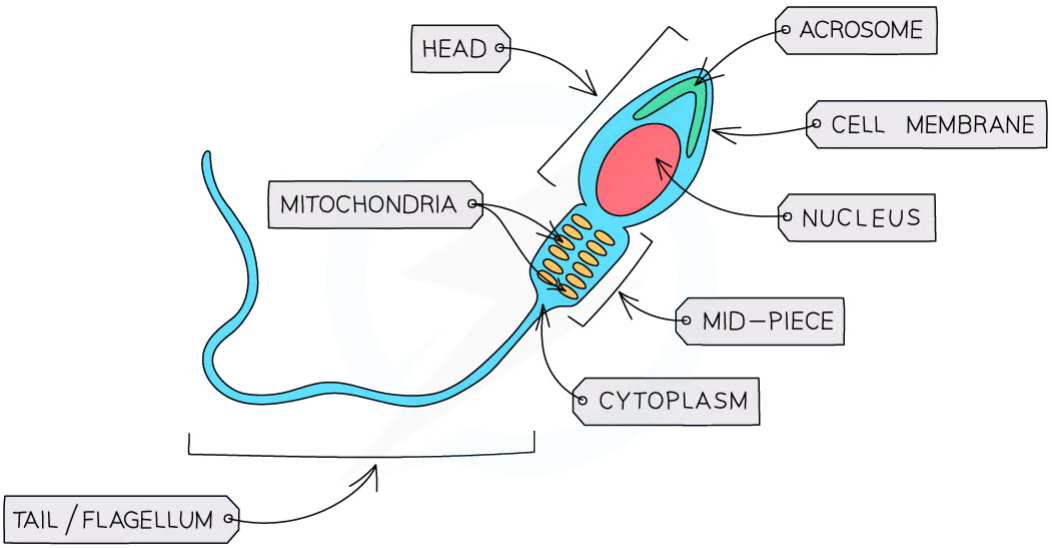
- Mendel was not the first to investigate inheritance using plants
- However, he was the first to generate strong numerical (**quantitative**) data, as opposed to observations only (**qualitative**)
- Mendel also used a **large number** of pea plants in his studies, for example in one investigation he recorded the characteristics for over 7,000 pea seeds
- By recording data for such a large number of seeds he ensured **reliability** in his conclusions
- **Many replicates** are important in a scientific investigation because they allow for:
  - Ensuring reliability of results
  - Identification of anomalous points
  - Further statistical analysis to establish a significant difference

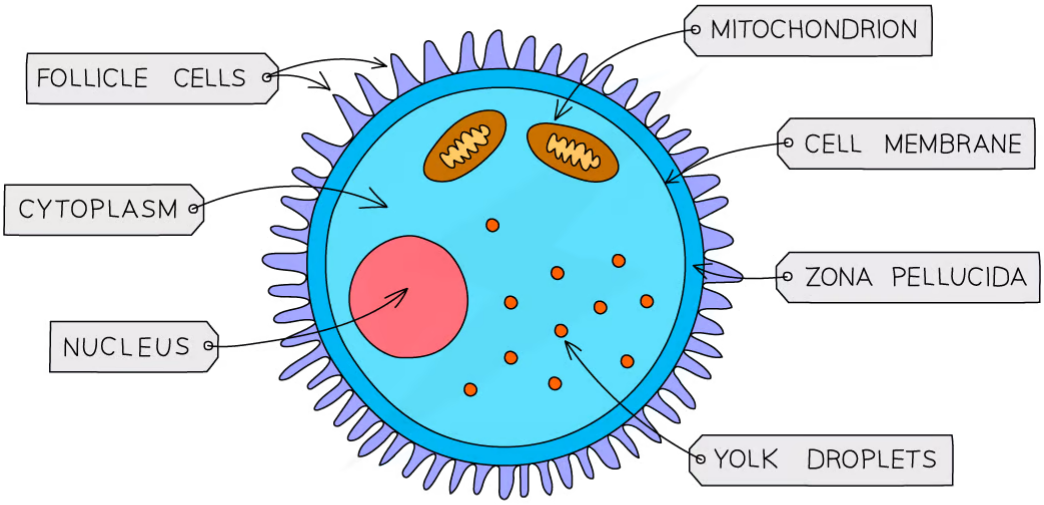
Gametes

YOUR NOTES

- Gametes are the **sex cells** of an organism
- For example, the **sperm** and **egg** (ovum) cells in humans
- The **egg is larger than the sperm** as most of its space contains food to nourish a growing embryo
- The sperm cell contains **many mitochondria** to release energy for its motion
- Gametes **fuse** during fertilization to form a **zygote**
- These sex cells are formed during **meiosis** and only have **one** copy of each chromosome and so are **haploid** cells
    - For humans, that means the sperm and egg cells contain **23 single chromosomes** in their nucleus
    - As there is only **one chromosome from each homologous pair** there is only **one allele** of each gene present
        - This allele may be dominant, recessive or **co-dominant**

YOUR NOTES





The structure of human gametes - the sperm and egg

Exam Tip

You will not need to be able to quote all of Mendel's experiments and his results but you should be able to explain why there is always a 3:1 ratio in second generation organisms

YOUR NOTES

Segregation of Alleles

- Meiosis is a form of **nuclear division** that results in the **production of haploid cells** from diploid cells
- During meiosis a diploid cell will **divide twice** to form **four haploid cells**
- It produces **gametes** in plants and animals that are used in **sexual reproduction**
- A diploid nucleus will contain **two** copies of each gene
- A haploid nucleus contains just **one** copy of each gene
- A diploid cell of genotype Yy will produce **two gametes carrying the Y allele** and **two carrying the y allele**
- The separation of alleles into different cells during meiosis is called **segregation**
- Segregation is important as it allows for **new allele combinations** in offspring

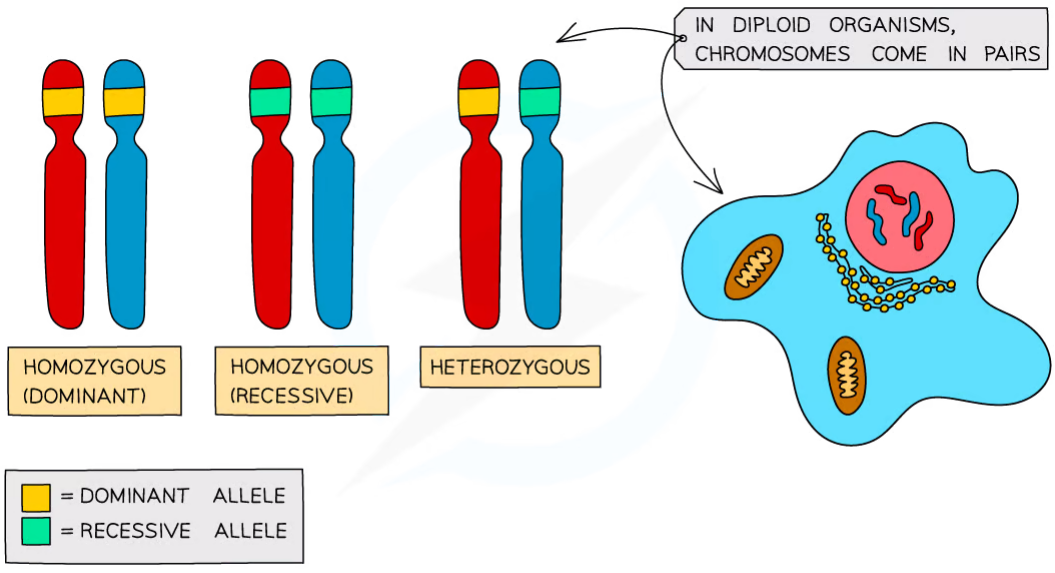
Diploid Zygotes

- Fusion of gametes results in diploid zygotes with two alleles of each gene that may be the same allele **or** different alleles
- Sexual reproduction is **a process involving the fusion of the nuclei of two gametes (sex cells) to form a zygote (fertilized egg cell) and the production of offspring that are genetically different from each other**
- Fertilization is defined as the **fusion of gamete nuclei**, and as each gamete comes from a different parent, there is **variation** in the offspring
- When a male and female gamete fuse their **chromosomes are combined**
- This means the resulting zygote is **diploid**
- The zygote contains two chromosomes of each type
- It will therefore also have **two alleles** of each gene
    - If the two alleles for a particular gene are the same then the **genotype** is described as **homozygous**
    - If the two alleles for a particular gene are different then the genotype is described as **heterozygous**

YOUR NOTES

Dominant, Recessive & Co-Dominant Alleles

- A **gene** is a short length of DNA found on a chromosome that codes for a particular **characteristic** (by coding for the production of a specific protein)
- **Alleles** are variations of the same gene
  - As we have two copies of each chromosome, we have two copies of each gene and therefore two alleles for each gene
  - One of the alleles is inherited **from the mother** and the other **from the father**
  - This means that the **alleles may not be the same**
  - For example, an individual has two copies of the gene for eye colour but **one allele could code for brown eyes and one allele could code for blue eyes**
- The **observable characteristics** of an organism (seen just by looking - like eye colour, or found – like blood type) is called the **phenotype**
- The **combination of alleles that control each characteristic** is called the **genotype**
- Alleles can be **dominant** or **recessive**
  - A dominant allele **only needs to be inherited from one parent** in order for the characteristic to be expressed in the phenotype
  - A recessive allele needs to be **inherited from both parents in order** for the characteristic to be expressed in the phenotype.
  - If there is only one recessive allele, it will **remain hidden** and the dominant characteristic will show
- If the two alleles of a gene are the same, we describe the individual as being **homozygous** (homo = same)
- An individual could be **homozygous dominant** (having two copies of the dominant allele), or **homozygous recessive** (having two copies of the recessive allele)
- If the two alleles of a gene are different, we describe the individual as being **heterozygous** (hetero = different)



YOUR NOTES

Alleles are different forms of the same gene. You can only inherit two alleles for each gene, and they can be the same (homozygous) or different (heterozygous). Alleles can be dominant or recessive.

- **Co-dominant alleles** have a combined effect on the phenotype
  - Certain **red-flowered plants** can be crossed with **white-flowered plants** of the same species, and the offspring's flowers have a **pink colour**
  - **Speckled chickens** show co-dominance between an **allele for white feathers** and an allele that causes the feathers to be **black**
  - The alleles are **both expressed to an equal extent** in the phenotype
- When completing genetic diagrams (punnet square diagrams), alleles are abbreviated to single letters
  - The dominant allele is given a **capital letter** and the recessive allele is given the same letter, but **lower case**
  - For example a tall (phenotype) pea plant can have the genotype **TT** or **Tt**; in this case 'T' represents the dominant tall allele and 't' represents the recessive dwarf allele

YOUR NOTES

Inheritance of Blood Groups

- Inheritance of blood group is an example of **co-dominance**
- This is of critical importance when deciding to give **blood transfusions** following injury or illness
- Use of the wrong blood group can cause an immune response that coagulates (solidifies) blood, leading to clots and serious illness/death
- There are **three** alleles of the gene controlling a person's blood group instead of the usual two
  - I represents the **gene** and the superscripts A, B and O represent the **alleles**
- Alleles I^A and I^B are **codominant**
- I^O is **recessive**
- I^A results in the production of **antigen A** on the surface of red blood cells
- I^B results in the production of **antigen B** on the surface of red blood cells
- I^O results in **no antigens** being produced on the surface of red blood cells
- These three possible alleles can give us the following genotypes and phenotypes

Blood Genotype & Phenotype Table

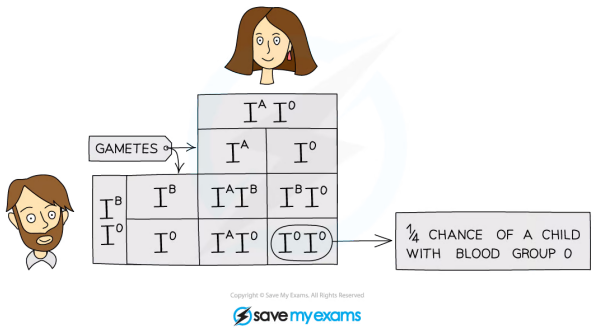
Genotype	Phenotype
I^AI^A or I^AI^O	A
I^BI^B or I^BI^O	B
I^AI^B	AB
I^OI^O	O

- We can use genetic diagrams to predict the outcome of crosses that involve the codominant alleles controlling blood groups

Worked Example

Show how a parent with blood group A and a parent with blood group B can produce offspring with blood group O.

YOUR NOTES



Punnett square showing the inheritance of blood group

Exam Tip

Take good care when hand-writing ABO blood group genotypes and alleles. The expected notation for ABO blood group alleles is that the letter I is always written in UPPERCASE and all the alleles must be in superscript. An example is I^AI^O

YOUR NOTES

Constructing Punnett Grids

- A **monohybrid trait** is one that is controlled by only **one gene**
- Generally, we consider that such a gene has **two alleles**
  - Either: one allele is dominant and the other is recessive
  - Or: the alleles are co-dominant

- A monohybrid cross starts with **pure-breeding parents** (homozygous), each displaying a different phenotype
- The **purpose of a Punnett grid** is to predict the probability of a certain offspring displaying a certain genotype or phenotype
  - In the case where multiple offspring are produced, Punnett grids can **predict the numbers of offspring** that will display a certain genotype or phenotype after a cross

Steps in constructing a Punnett Grid

1. Write down the **parental phenotypes** and **genotypes**
2. Write down all the **possible gamete genotypes** that each parent could produce for sexual reproduction
   - A useful convention is to write the gamete genotypes **inside a circle** to denote them as gametes (haploid cells)
3. Place each parental genotype **against one axis** of a Punnett grid (2 x 2 table)
4. In the boxes of the Punnett grid, combine the gametes into the possible genotypes of the offspring
   - This gives the offspring of the **F_1 generation** (1^{st} filial generation)
5. List the **phenotype** and **genotype ratios** for the offspring

Worked Example

Sweet peas grow pods that are either green or yellow. The allele for green, **G**, is dominant to the allele for yellow, **g**.Construct a Punnett grid to predict the outcome when crossing green and yellow pure-bred plants to show the F_1 generation offspring.Using plants from the F_1 generation, construct a second Punnett grid to show the outcomes of the F_2 generation.

Step 1: Write down the parental phenotype and genotypes

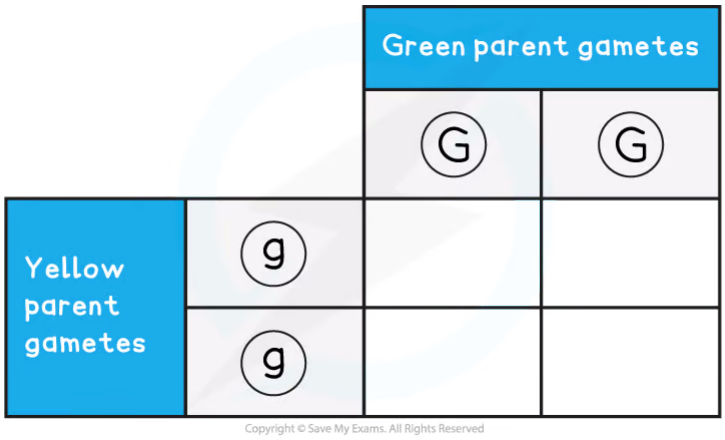
Green coloured pods	Yellow coloured pods
GG	gg

Step 2: Write down all the possible gamete genotypes that each parent could produce

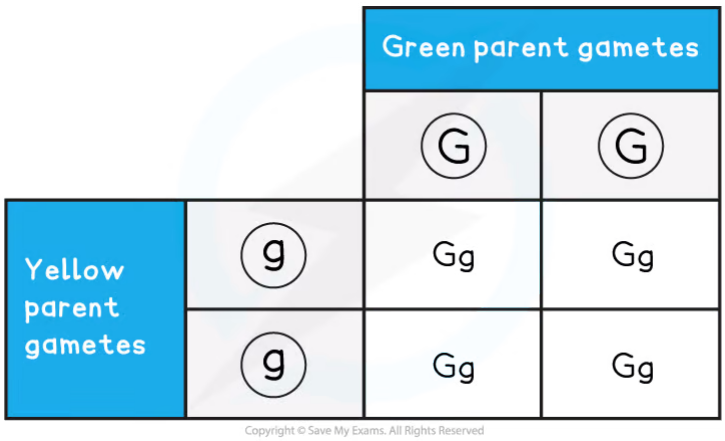
(G) (g)

Step 3: Place each parental genotype against one axis of a Punnett grid (2 x 2 table)

YOUR NOTES

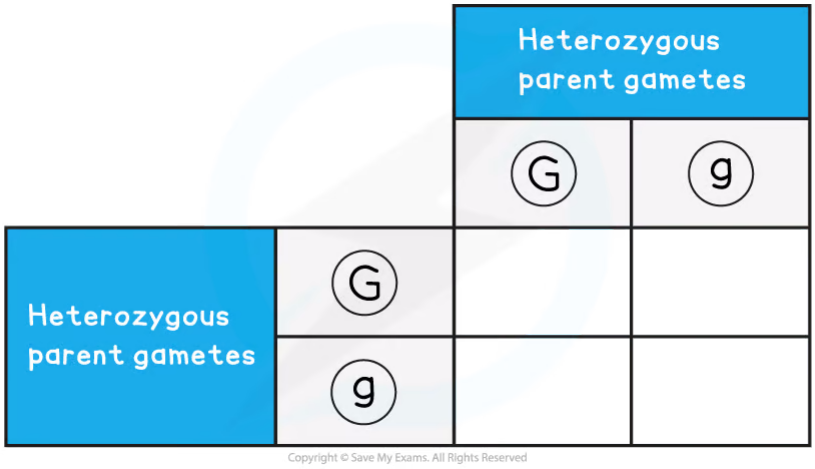


Step 4: Combine the gametes in each box of the Punnett grid



Genotypes of the F1 cross between homozygous green (GG) and homozygous yellow (gg) pea plants. All offspring (100%) have the genotype Gg and the phenotype is green.

Step 5: Take two heterozygous offspring from the F_1 generation and cross them



Step 6: Combine the gametes in each box of the Punnett grid

YOUR NOTES

		Heterozygous parent gametes	
		G	g
Heterozygous parent gametes	G	GG	Gg
	g	Gg	gg

Punnett grid showing the results of the F2 generation

Phenotype ratio is 3:1 green:yellow, Genotype ratio is 1 GG: 2 Gg: 1 gg

Analysis of Genetic Crosses

YOUR NOTES

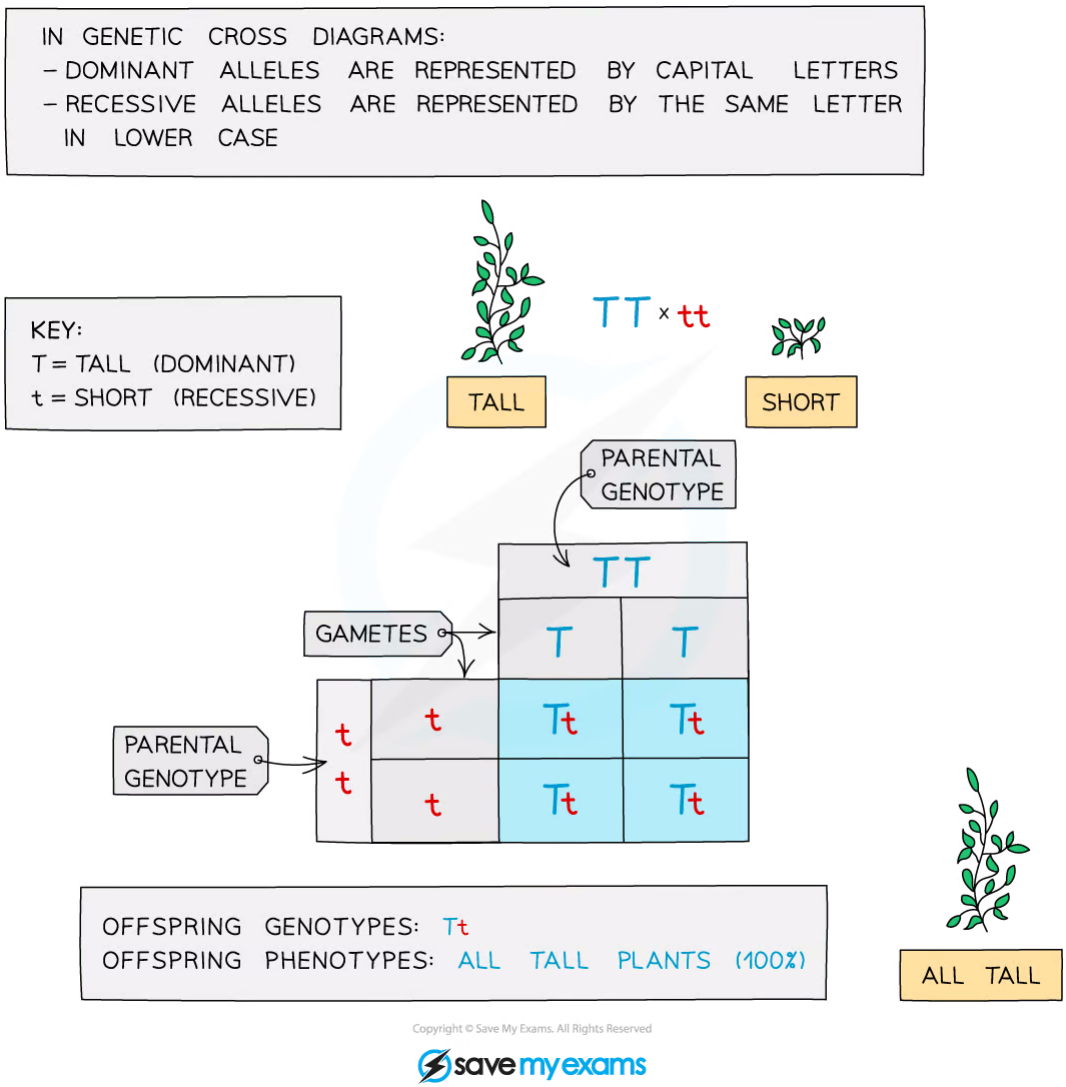
Comparison of predicted and actual outcomes of genetic crosses using real data

- A Punnett grid diagram shows the **possible combinations of alleles** that could be produced in the offspring of a certain genetic cross
- From this, we can deduce the **expected ratio** of these combinations
- The actual outcome achieved from the cross often presents **ratios different** to those deduced in the punnet grid
- This is because there is an element of **chance** involved with inheritance
- The **chi-squared test** is a statistical test that can be used to determine whether the differences between the observed ratios and the expected ratios are **significant or due to chance**

Using pea plants to demonstrate the predicted outcomes of genetic crosses

- The height of pea plants is controlled by a single gene that has two alleles: tall and short
- The tall allele is dominant and is shown as **T**
- The small allele is recessive and is shown as **t**
- A pure breeding short plant is bred with a pure breeding tall plant
- The term '**pure breeding**' indicates that the individual is homozygous for that characteristic

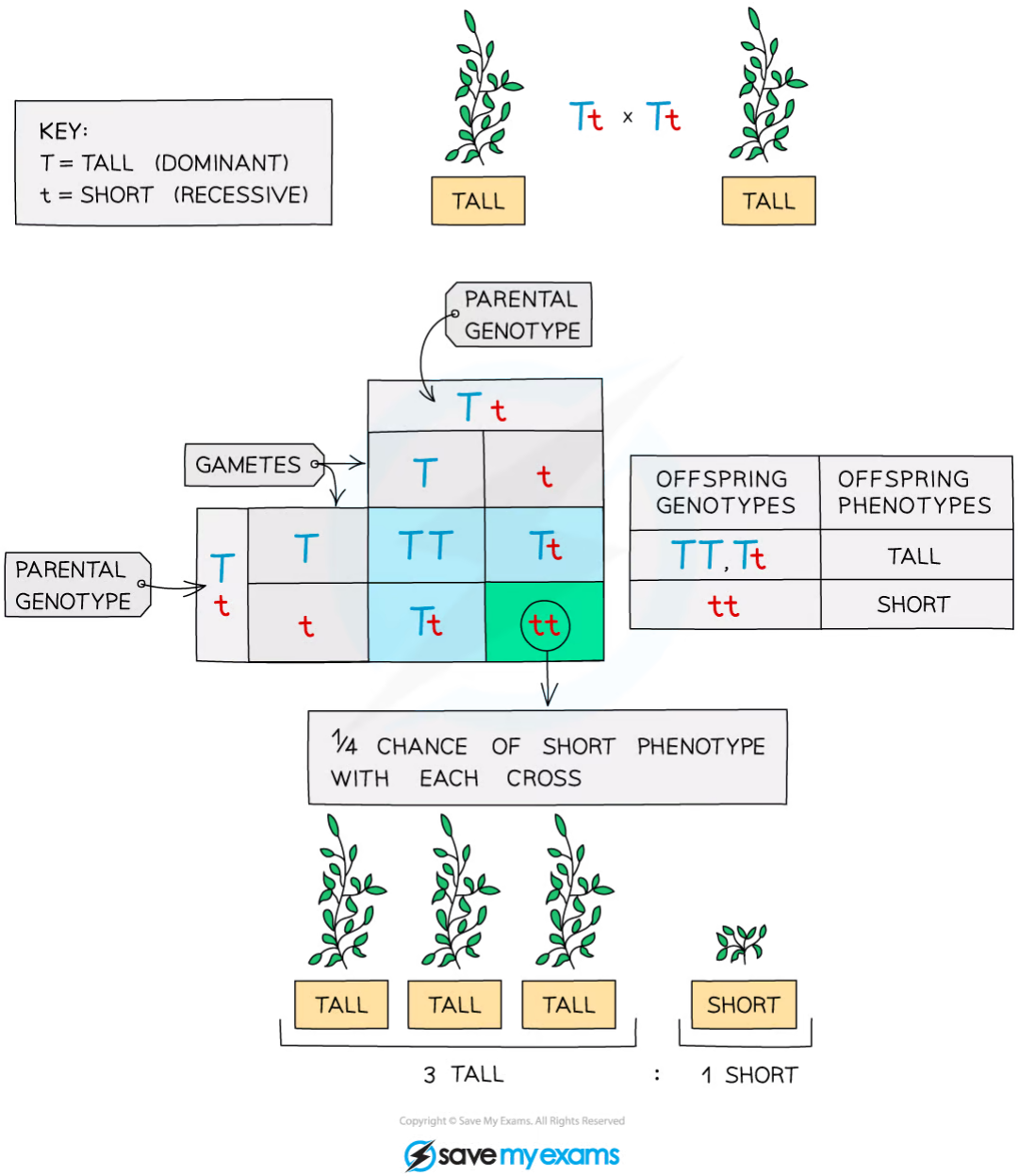
YOUR NOTES



A pure-breeding genetic cross in pea plants. It shows that all offspring will be have the tall phenotype.

- Crossing the offspring from the first cross

YOUR NOTES



A genetic cross diagram (F2 generation). It shows a ratio of 3 tall : 1 short for any offspring.

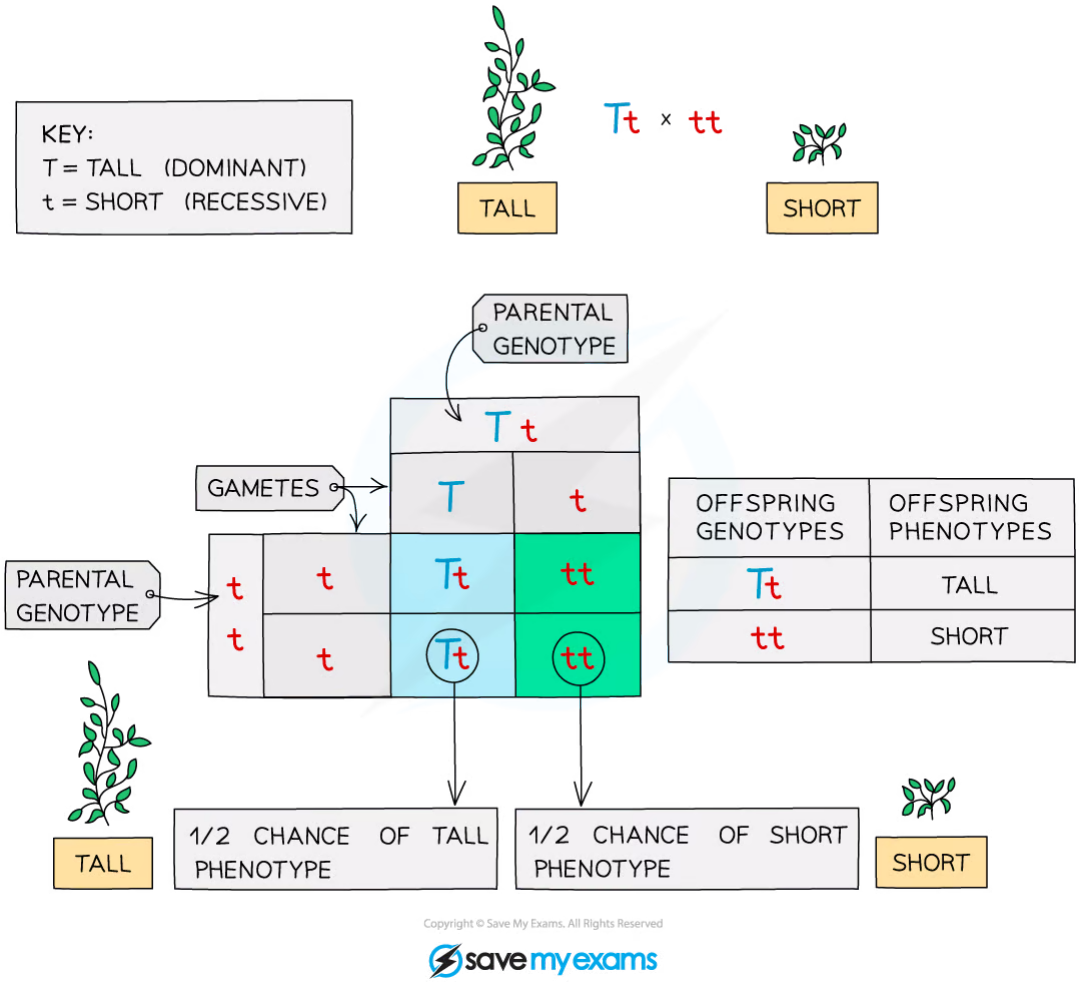
- All of the offspring of the first cross have the same genotype, **Tt** (heterozygous), so the possible combinations of offspring bred from these are: **TT** (tall), **Tt** (tall), **tt** (short)
- There is more variation in the second cross, with a **3:1** ratio of tall : short
- The F2 generation is produced when the offspring of the F1 generation (pure-breeding parents) are allowed to interbreed

Crossing a heterozygous plant with a short plant

- The heterozygous plant will be tall with the genotype **Tt**
- The short plant is showing the recessive phenotype and so must be homozygous recessive – **tt**

YOUR NOTES

- The results of this cross are as follows:



A cross between a heterozygous plant with a short plant

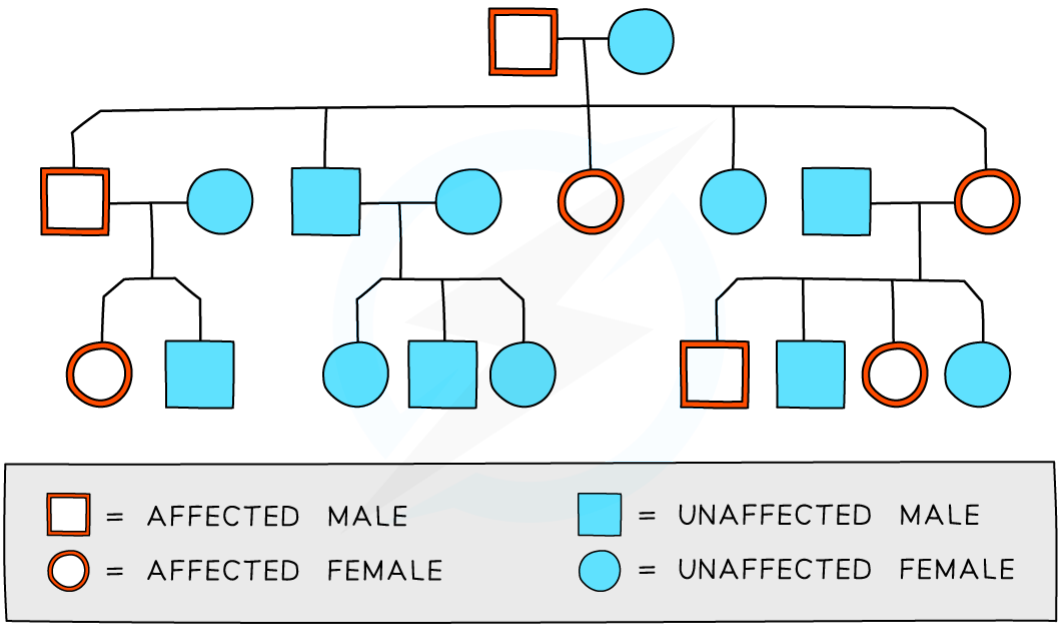
Exam Tip

If you are asked to use your own letters to represent the alleles in a Punnett grid, try to choose a letter that is obviously different as a capital than the lower case so the examiner is not left in any doubt as to which is dominant and which is recessive.

Pedigree Charts

YOUR NOTES

- Family pedigree diagrams are usually used to trace the **pattern of inheritance** of a specific characteristic (usually a disease) **through generations of a family**
- This can be used to work out the probability that someone in the family will inherit the **genetic disorder**



A family pedigree chart

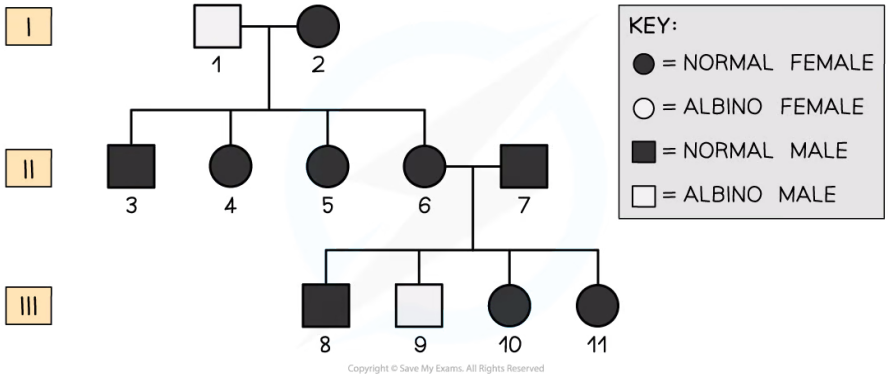
- Males are indicated by the **square shape** and females are represented by **circles**
- In this diagram, affected individuals are **red** and unaffected are **blue**
  - **Shading or cross-hatching** may also be used to show affected individuals
- **Horizontal lines** between males and females show that they have **produced children** (which are linked underneath each couple)
- Roman numerals may be used to indicate **generations**
- For each generation the eldest child is on the left and **each individual** is **numbered**
- The family pedigree above shows:
  - both males and females are affected
  - every generation has affected individuals
  - The eldest son (in the second generation) is affected
  - That there is one family group that has no affected parents or children
  - the other two families have one affected parent and affected children as well

YOUR NOTES

Worked Example

Worked example: Pedigree charts

- Below is a pedigree chart which traces the inheritance of **albinism** across several generations. Albinism affects the production of the **pigment melanin** leading to lighter hair, skin and eyes.



- Using the pedigree chart, deduce and explain the following:

1. What type of allele causes albinism
2. The genotype of individuals named **9** and **7**
3. The possible genotypes of **10** and **11**

1. Albinism is caused by a **recessive allele**
   - Explanation: We can tell this from the pedigree chart because expression of the disease skips generation II. Also, person number 9 is an affected individual despite his parents (6 and 7) being unaffected. 6 and 7 must both be carriers of the recessive allele and 9 has inherited one recessive allele from each parent.
   - It is unlikely to be a **sex-linked disease as both females and males** have the condition

2. The genotype of person 9 must be **homozygous recessive** (aa) and the genotype of 7 must be **heterozygous** (Aa)
   - Explanation: 9 is an affected individual with albinism (which is determined by the recessive allele). 7 must be heterozygous in order for him to pass on the recessive allele to person 9

3. The possible genotypes of 10 and 11 are **heterozygous** (Aa) or **homozygous dominant** (AA)
   - Explanation: This is because they are unaffected individuals so must possess at least one dominant allele (A), however, it is possible that they each inherited a dominant allele from each parent

Exam Tip

When answering questions about pedigree charts for genetic diseases, it is always useful to remember which phenotype is caused by the recessive allele. You can write these genotypes onto your chart and it will give you a good starting point for working out the possible genotypes of the rest of the individuals in the chart.

YOUR NOTES

Causes of Genetic Diseases

- A gene can affect the phenotype of an organism
  - A gene codes for a single polypeptide
  - The polypeptide affects the phenotype through a particular mechanism
- The phenotype of an individual can also be affected by the **environment**
- A **genetic disease** is caused by a gene which results in an abnormal protein that **alters the phenotype** of the individual
- Most genetic diseases are caused by recessive alleles on autosomal chromosomes
  - This means that an individual would need **two** copies of the recessive allele in order to develop the disease
  - Individuals that are heterozygous do not suffer from the disease but are **carriers** and can pass the recessive allele on to the **next generation**
  - A disease determined by a recessive allele includes **cystic fibrosis**
- Some diseases are caused by dominant
  - This means that only **one** copy of the allele is required in order to develop the disease and this one copy can also be passed on to the next generation
  - Individuals that are homozygous dominant, **will suffer from the disease** and will also pass the allele on to the next generation with **100%** probability
  - A disease determined by a dominant allele includes **Huntington's disease**
- It is also possible, but rare, for a disease to be caused by codominant alleles
  - This means that in individuals with heterozygous genotype, **both alleles** are expressed in the phenotype
  - Therefore giving a **3rd phenotype** that is different to the homozygous phenotypes
  - A disease determined by codominant alleles includes **sickle cell anaemia**
- The genes which causes some genetic diseases are found on the **sex chromosomes**
  - This means they affect males and females **differently**
  - Examples of sex-linked diseases include **haemophilia** and **colour blindness**

YOUR NOTES

Exam Tip

You may be asked to predict the inheritance of diseases like the ones above. An example question would be:

Max and Jane are trying for a baby but they are concerned about the possibility of their child having haemophilia. Neither Max or Jane have haemophilia themselves but Jane's father had the condition. What are chances that their child could have haemophilia?

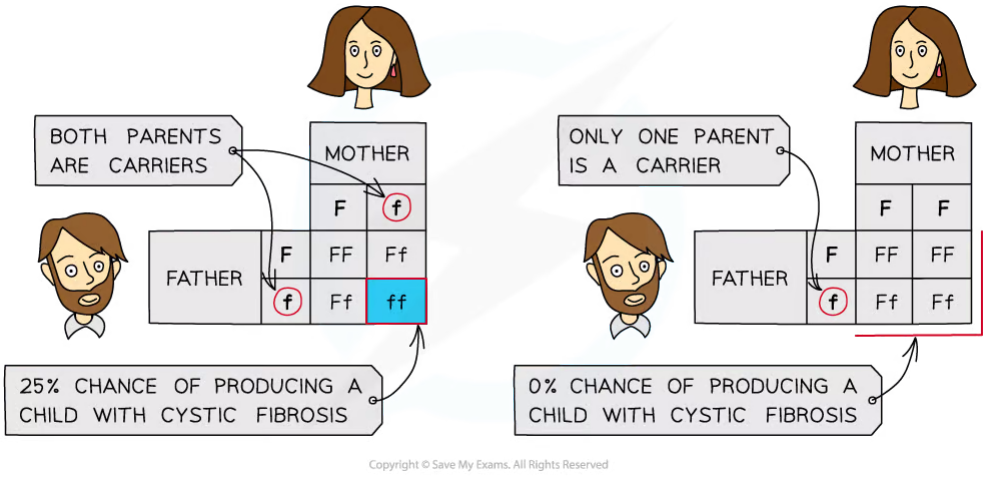
For questions like this, it is very important to gather early on whether the abnormal allele that causes the disease is dominant or recessive and if there is any sex linkage. In this example for haemophilia, the abnormal allele is recessive and the gene is sex-linked. Then the next step would be to work out the genotypes of the parents from the information given and use this to create a genetic diagram.

Examples of Genetic Diseases

YOUR NOTES

Cystic Fibrosis

- Cystic fibrosis is a genetic disorder of **cell membranes** caused by a **recessive** allele (**f**) of the CFTR gene located on chromosome 7
  - This gene codes for the production of chloride ion channels required for secretion of sweat, mucus and digestive juices
- A fault in the CFTR gene leads to production of **non-functional channels**
- This leads to reduced levels of **sodium chloride** in secretions which subsequently reduces the movement of **water by osmosis** into the secretions
- Ultimately resulting in the body producing large amounts of thick, sticky mucus in the air passages
- Over time, the mucus builds up in the **lungs** leading to infections and blocks narrow passage ways, such as the **pancreatic duct,** leading to reduced digestive efficiency as a result of less enzyme secretions
- Cystic fibrosis is determined by a recessive allele, this means:
  - People who are **heterozygous** (only carry one copy of the recessive allele) won't be affected by the disorder but are **'carriers'**
  - People must be **homozygous recessive** (carry two copies of the recessive allele) in order to have the disorder
  - If both parents are carriers, the chance of them producing a child with cystic fibrosis is 1 in 4, or 25%
  - If only one of the parents is a **carrier** (with the other parent being homozygous dominant), there is no chance of producing a child with cystic fibrosis



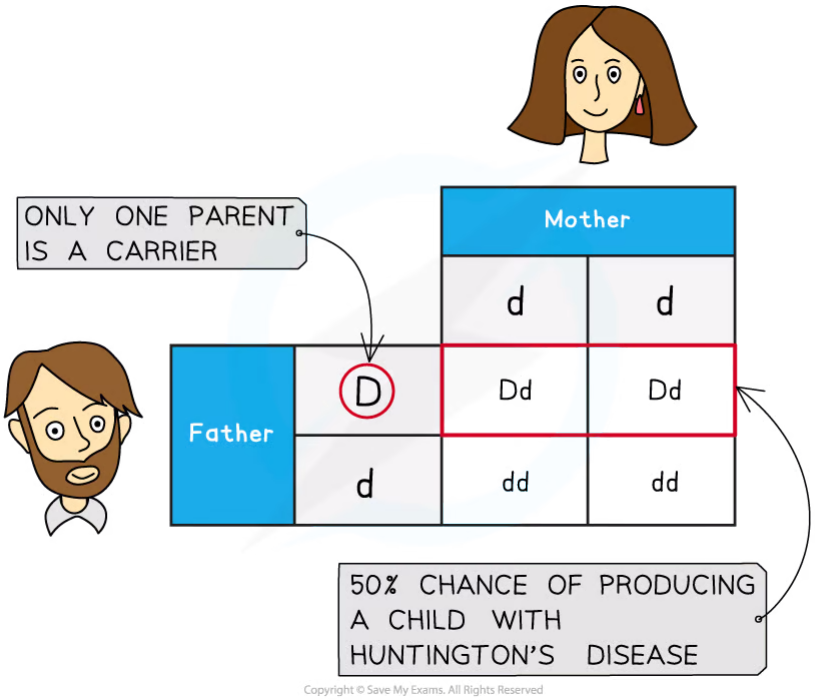
Inheritance of cystic fibrosis if both parents are carriers or if only one parent is a carrier

Huntington's Disease

- Huntington's disease is a genetic condition that develops as a person ages
- Usually a person with the disease will not show symptoms until they are 30 plus years old
- An individual with the condition experiences **neurological degeneration**; they lose their ability to walk, talk and think
- The disease is ultimately fatal

YOUR NOTES

- It has been found that individuals with Huntington's disease have **abnormal alleles of the *HTT* gene**
  - The *HTT* gene codes for the protein **huntingtin** which is involved in neuronal development
- The **abnormal allele is dominant** over the normal allele
  - If an individual has one abnormal allele present they will suffer from the disease
  - If only one parent is a carrier of the dominant allele, there is still a 1 in 2 or 50% chance of producing a child with the disease



Huntington's is caused by a dominant allele

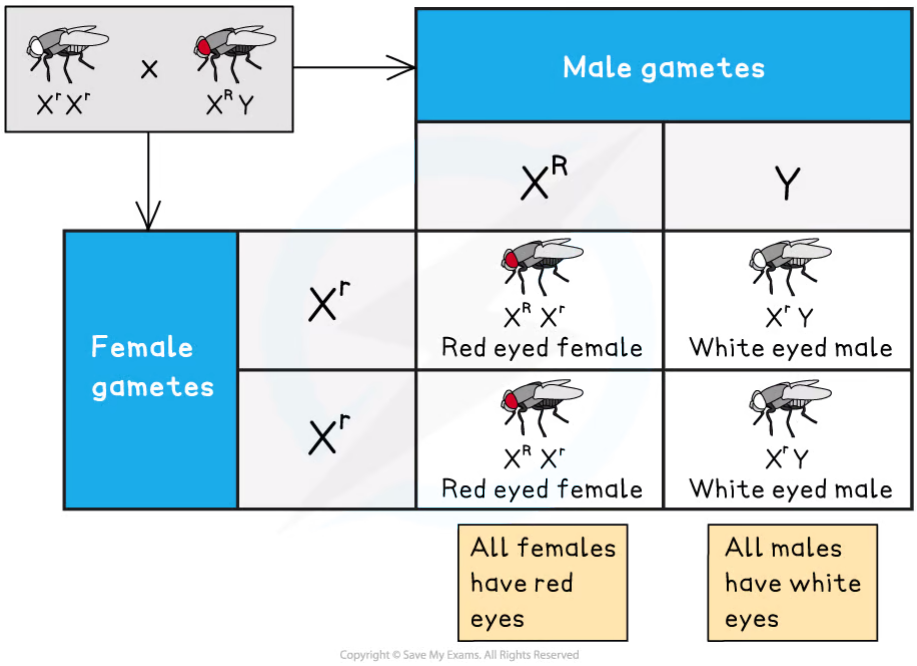
YOUR NOTES

Sex-linked Genetic Diseases

- Some genetic diseases in humans are sex-linked
- Inheritance of these diseases is different in males and females
  - Sex-linked genes are only **present on one sex chromosome and not the other**
  - This means the sex of an individual affects what alleles they pass on to their offspring through their gametes
- If the gene is on the X chromosome **males (XY) will only have one copy** of the gene, whereas females (XX) will have two
- There are **three phenotypes** for **females** - normal, carrier and has the disease, whereas **males** have only **two** phenotypes – normal or has the disease

Sex linkage in drosophila

- Thomas Morgan discovered sex linkage in *Drosophila* through studying the **ratios** of offspring produced from crosses of flies with different **eye colours**
- He found that when crossing white eyed females and red eyed males, the ratios achieved were different to those observed when crossing red eyed females with white eyed males
- For example, when crossing a red eyed male, X^RY, with a white eyed female, X^rX^r, Thomas Morgan found that all female offspring had red eyes and all male offspring had white eyes
  - If this was a gene found on an autosome, he would have expected to see that **all** of the first filial (F_1) generation would have red eyes because red is dominant
- Analysis of the results lead Thomas Morgan to deduce that the gene for eye colour was found on the **X chromosome** and therefore males possessed only **one** allele for that phenotypic characteristic



Eye colour in Drosophila is determined by a gene found on the sex chromosomes

Exam Tip

The expected notation when writing about sex linked alleles is to use upper case 'X' and 'Y' for the chromosome, next to superscript letters to represent the allele. For example X^fX^f or X^fY.

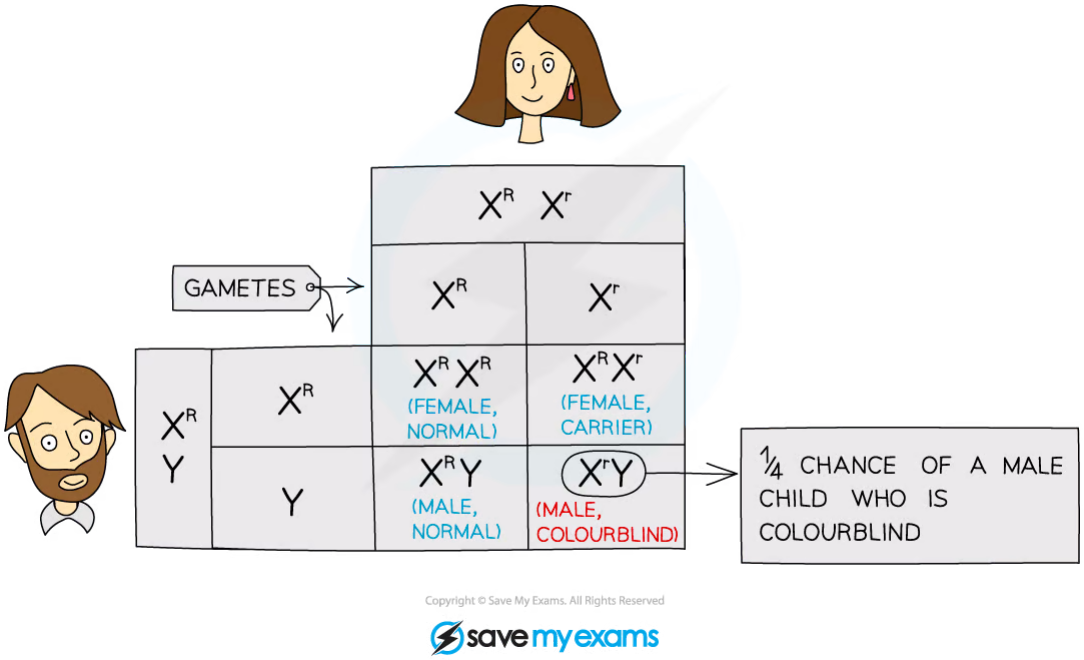
YOUR NOTES

YOUR NOTES

Examples of Sex-Linked Genetic Diseases

Red-green colour blindness

- The gene which is responsible for synthesizing the **photoreceptor proteins** of the eye, are found on the **X chromosome**
- The photoreceptor proteins are made in the cone cells of the eye and detect the specific **wavelengths** of light entering the eye
- Red-green colour blindness is caused by a **recessive allele** of this gene
- Males are more likely to be red-green colour blind as they only posses 1 allele for the gene, whereas females have 2 alleles and need to inherit 1 faulty allele from both parents in order to be colour blind



Punnett grid showing the inheritance of colourblindness, an X-linked condition

Haemophilia

- Haemophilia is a well known sex-linked disease
- There is a gene **found on the X chromosome** that codes for a protein called factor VIII. Factor VIII is needed to make blood clot
- There are two alleles for factor VIII, the dominant **F** allele which codes for normal factor VIII and the recessive **f** allele which results in a lack of factor VIII
- When a person possesses only the recessive allele **f**, they don't produce factor VIII and their blood can't clot normally
- If males have an abnormal allele they will **have the condition** as they have only one copy of the gene
- Females can be heterozygous for the faulty gene and not suffer from the condition but act as a **carrier**
- This means that haemophilia is a potentially fatal genetic disease which affects males more than females

YOUR NOTES

Worked Example

Worked example: Haemophilia

- The genetic diagram below shows how two parents with normal factor VIII can have offspring with haemophilia

Parental phenotypes: carrier female x normal male

Parental genotypes: X^FX^f X^FY

Parental gametes: X^F or X^f X^F or Y

Monohybrid Punnett Square with Sex-linkage Table

		Male gametes	
		X^F	Y
Female gametes	X^F	X^FX^F / female with normal blood clotting	X^FY / male with normal blood clotting
	X^f	X^FX^f / carrier female	X^fY / male with haemophilia

Predicted ratio of phenotypes in offspring

1 female with normal blood clotting : 1 carrier female : 1 male with haemophilia : 1 male with normal blood clotting

Predicted ratio of genotypes in offspring: $1X^FX^F : 1X^FX^f : 1X^FY : 1X^fY$

Exam Tip

Make sure to include all of your working out when constructing genetic diagrams. It is not enough just to complete a punnett grid, you need to show that you have thought about the **possible gametes** that can be produced by each parent.Also, remember to state the **phenotype** as well as the genotype of the offspring that result from the cross. Read the questions carefully when answering sex-linked inheritance questions – is the question asking for a probability for **all** children or is it asking about a specific sex (males or females).

Human Genetic Diseases

YOUR NOTES

- There are **thousands** of genetic diseases that affect humans, although many are caused by recessive alleles and are therefore very **rare**
- Naturally numbers of people suffering from diseases caused by recessive allele remains very low due to the low probability of inheriting 2 recessive alleles. This is the recognised pattern of **Mendelian inheritance**
- Additionally, the **Human Genome Project** made it possible to **sequence** the entire human genome and opened up the opportunity for scientists to pinpoint which part of the genome is responsible for specific genetic diseases
- Now individuals can go through tests to sequence their own genome in order to establish the likelihood of inheriting an allele which could cause a genetic disease
- Parents may choose to go through this sequencing process before having children so that they are aware of the risks
    - They can make informed decisions based on the outcome of the test

YOUR NOTES

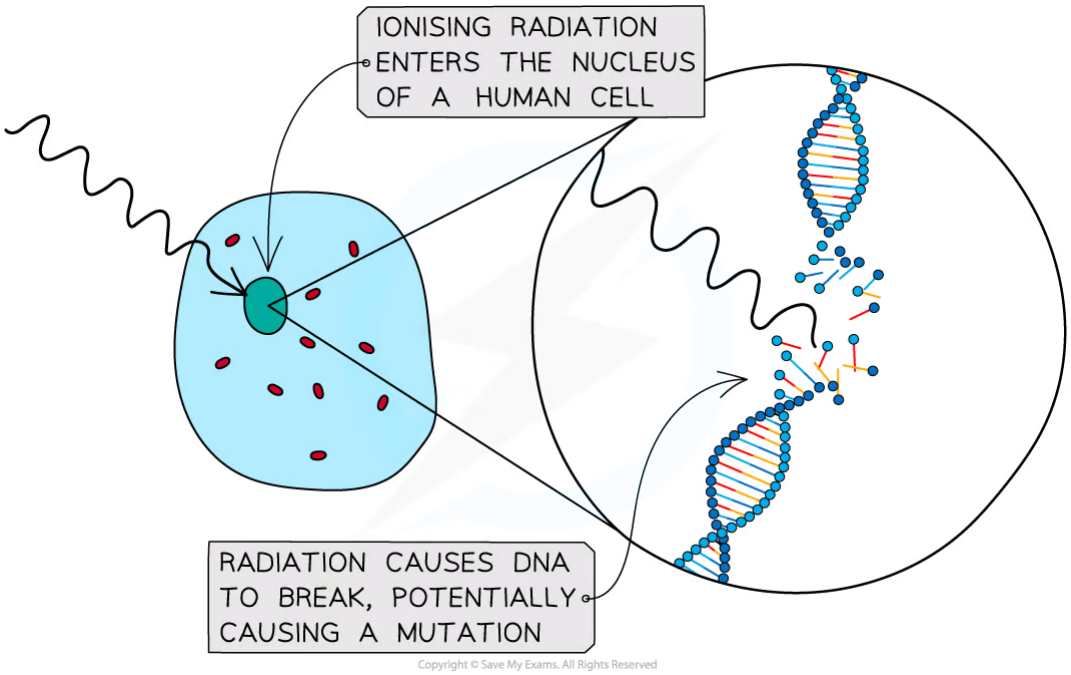
Causes of Mutations

- A **mutation** is a change in the sequence of base pairs in a DNA molecule
- They occur **randomly** and **continuously** to create new alleles of a gene
  - Often only with a very small number of differences in the base sequence
- As the DNA base sequence determines the sequence of amino acids that make up a polypeptide, **mutations in a gene can sometimes lead to a change in the polypeptide** that the gene codes for
  - If the change is significant, this could be harmful for the organism (mutations are rarely beneficial). It **may affect the ability of the protein to perform its function**
  - For example:
    - If the shape of the active site on an enzyme changes, the substrate may no longer be able to bind to the active site
    - A structural protein (like collagen) may lose its strength if its shape changes
- Most mutations are **neutral** because they **do not alter the polypeptide** or only alter it slightly so that its structure or function is not changed
  - This is because the genetic code is **degenerate**
- Mutations in body cells can lead to cancer. These mutations are often eradicated when the individuals dies
- Mutations of cells which are involved in gamete production can be **inherited** by the next generation

Mutagenic agents

- There are natural mechanisms that take place within cells to ensure the accuracy of **DNA replication**
  - These mechanisms involve proofreading and repairing damaged DNA
- When the mutation rate of a cell rises to above a normal (usually low) rate then these mechanisms have become ineffective
- Mutagenic agents are environmental factors that **increase the mutation rate of cells**
- **Radiation** can cause chemical changes in DNA, this includes:
  - **High-energy** radiation such as UV light
  - Ionising radiation such as X-rays, gamma rays and alpha particles
- **Chemical substances** can also caused changes to DNA, examples include
  - Benzo[a]pyrene and nitrosamines found in tobacco smoke
  - Mustard gas used as a chemical weapon in World War I

YOUR NOTES



Ionising radiation can cause mutations in DNA

Effects of Radiation

YOUR NOTES

Chernobyl Nuclear Disaster

- **Chernobyl** Nuclear Power Plant is in Ukraine
- In 1986 an incident at the plant caused an explosion and fire in the nuclear reactor core
- A large amount of radioactive material was released from the plant and went into the air
  - Radioactive isotopes of xenon, krypton, iodine, caesium, and tellurium were released as well as large amounts of small particles of uranium
- Hundreds of thousands of people were evacuated from the surrounding area to protect them from being contaminated
- An exclusion zone of around 2,600 square kilometres is still in place around the power plant
  - This is because the level of radiation in the area is still very high

The Chernobyl Disaster is probably the worst nuclear disaster in history

- The effects of the Chernobyl powerplant explosion were significant:
  - The total number of radiation related deaths to date has reached 4000
  - Large areas of pine forest **turned brown** and **died** in the weeks afterwards
  - **Agricultural animals** died due to **thyroid damage** caused by radioactive iodine and the **consumption** of contaminated meat e.g. lamb, was **banned**
  - **Milk** produced contained high levels of iodine in areas where waterways had been contaminated
  - **Bioaccumulation** of radioactive materials occurred in nearby waterways affecting fish in countries thousands of kilometres away and **contaminating drinking water** for many species
  - Thousands of cases of **thyroid cancer** were recorded as a direct result of the radioactive iodine, including over 4000 in children and adolescents

YOUR NOTES

- However, despite all this, there is **no significant evidence** of an increase in **solid cancers** or **leukaemia** even in the most affected population
- With no human habitation within the exclusion zone since the explosion, **other wild animals and many species of plants** have moved in and **colonised** the area
  - Despite the high radiation levels, the life expectancy of these organisms has not been shortened
- The long term effects on those who were exposed to **low levels** of radiation are yet unknown as studies continue

Nuclear bombing of Hiroshima and Nagasaki

- Two atomic bombs were dropped in Japan towards the end of World War II. One on Hiroshima and one on Nagasaki
- Between 150 000 and 200 000 people died as a direct result of the bombs
  - Half of these people died on the day the bombs were detonated and the rest died in the months immediately afterwards as a result of **burns, radiation sickness, injuries or through illnesses and malnutrition**
- Huge studies were carried out on other **survivors,** compared to a control group, to build a bigger picture of the longer term impact of high **exposure to radiation**
  - Incidence of cancer were much higher in the survivors studied compared to the control group
  - An **increase in leukaemia** cases was seen in both cities after a 2 year delay, which reached a peak around 6 years after the bombings
  - Those who were closer to ground zero seemed to be more seriously affected
  - There were also thousands of recorded **cancerous tumours** in the groups being monitored, although due to **confounding factors**, only around 800 could be formally attributed to the effects of radiation
- A large study was also carried out into the effects on **babies** pre and post birth
  - It was expected that there would be high numbers of mutations resulting in subsequent stillbirths or deformities, however, the numbers of incidence recorded were **not significant**
  - There was **no evidence** to suggest that babies conceived by survivors of the bombings were more likely to be born with birth defects
- There were many more **social impacts** associated with the bombs
  - The survivors were labelled 'Hibakusha', meaning 'the explosion affected people' and the associated stigma lead to widespread discrimination
    - There were **concerns** about whether the Hibakusha were **contagious** or whether the illnesses that they experienced were **heritable**. **Sterilisation** programs were even considered
    - As a result, many survivors struggled to find **employment or marry**

YOUR NOTES

3.4 Genetic Modification & Biotechnology

3.4.1 Electrophoresis & PCR

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

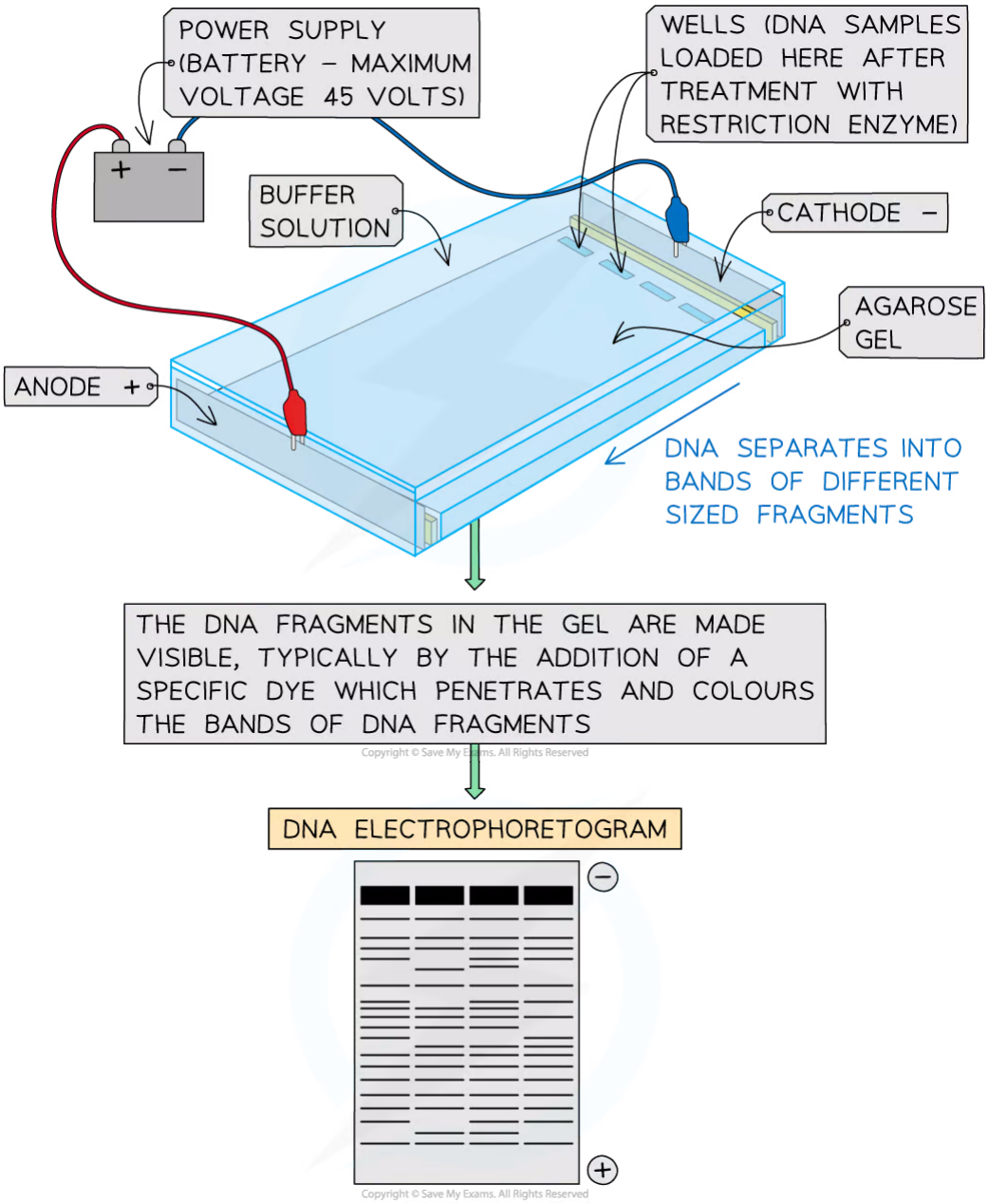
Method

- **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

YOUR NOTES

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



The process of electrophoresis

Exam 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

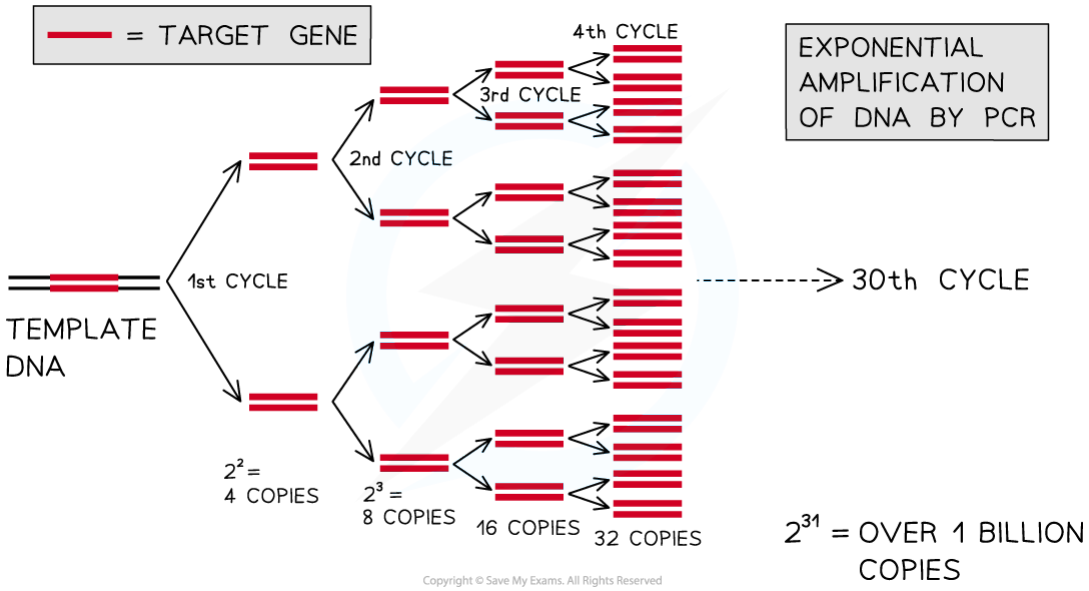
The key stages of PCR

- 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

YOUR NOTES

- **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

Exam 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

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

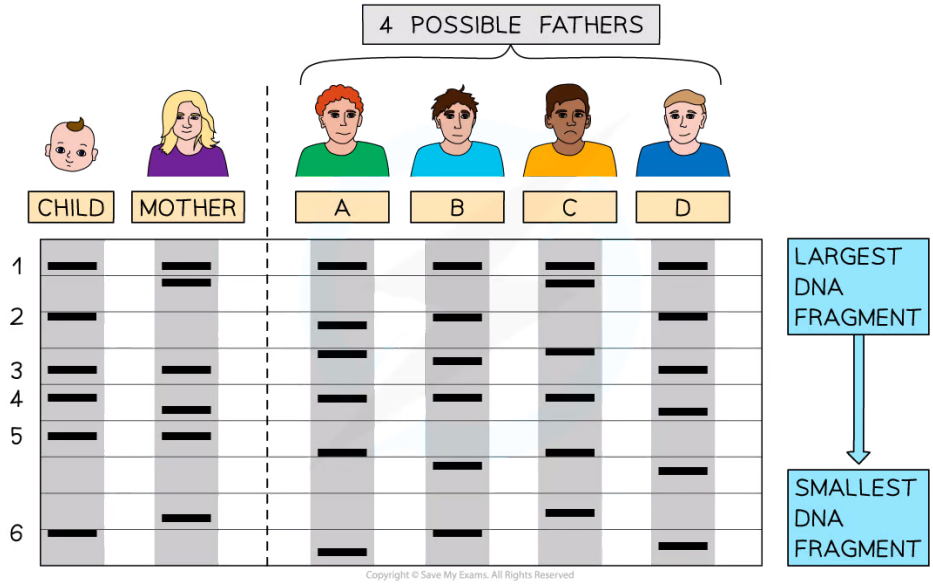
YOUR NOTES

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)

YOUR NOTES

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

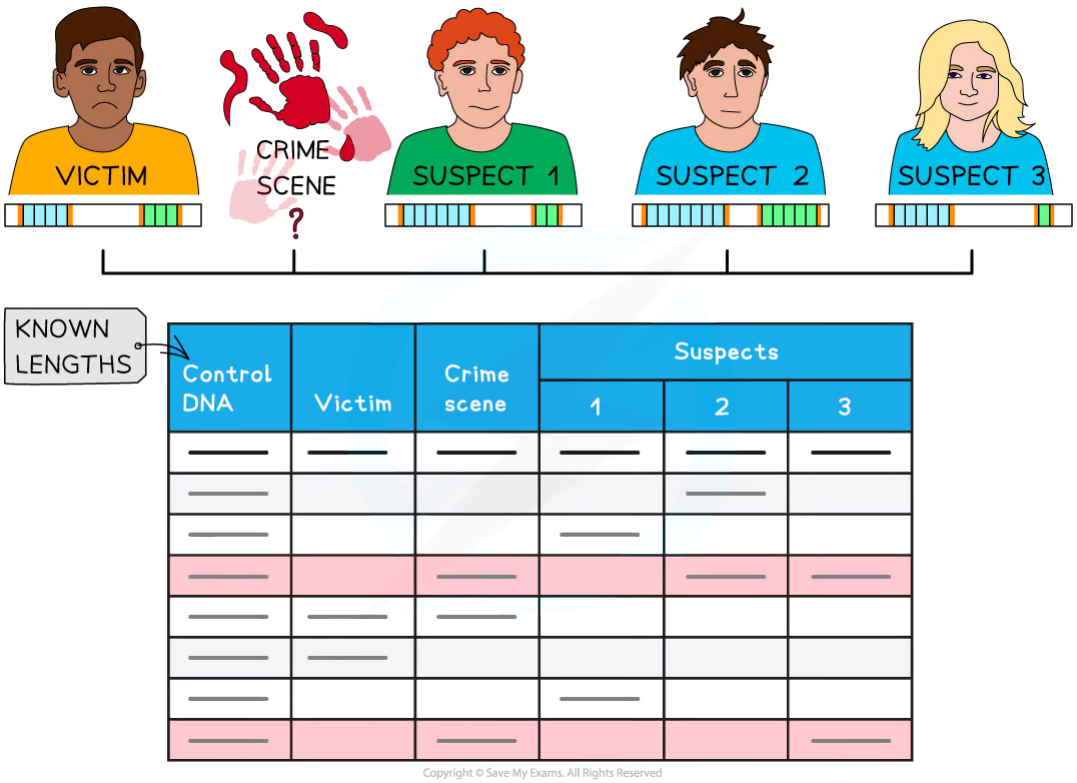
Step 6: Look for supporting evidence from band 6

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**

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.

Exam 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.

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

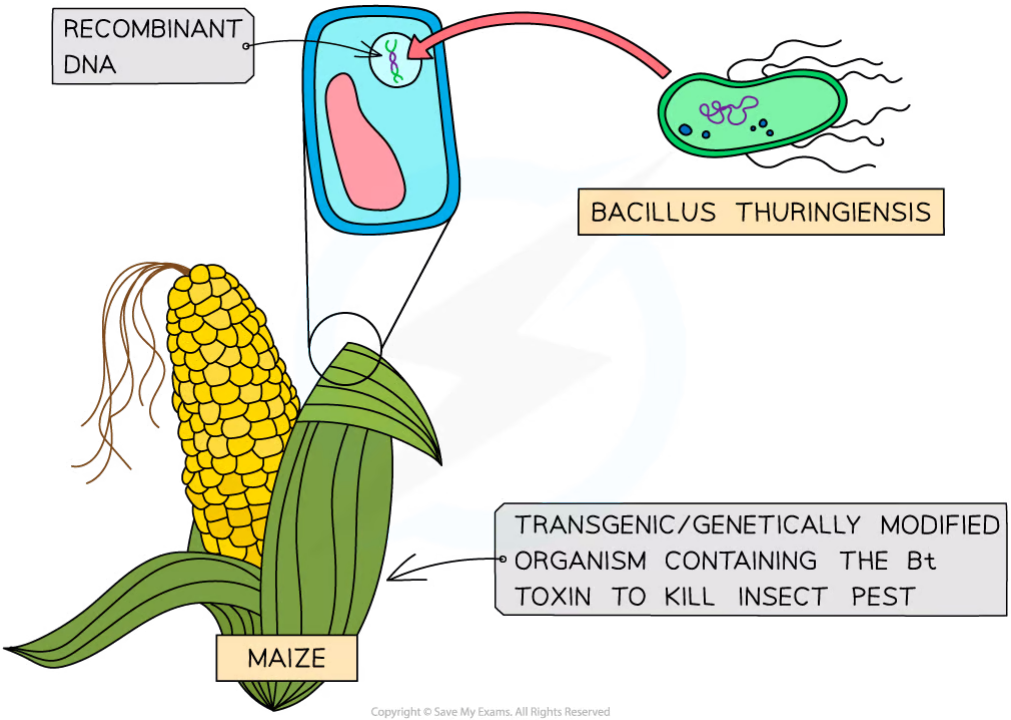


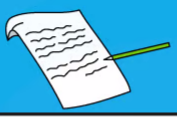
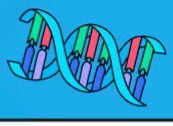
***Illustration of a maize plant that has recombinant DNA (DNA from* Bacillus thuringiensis)**

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

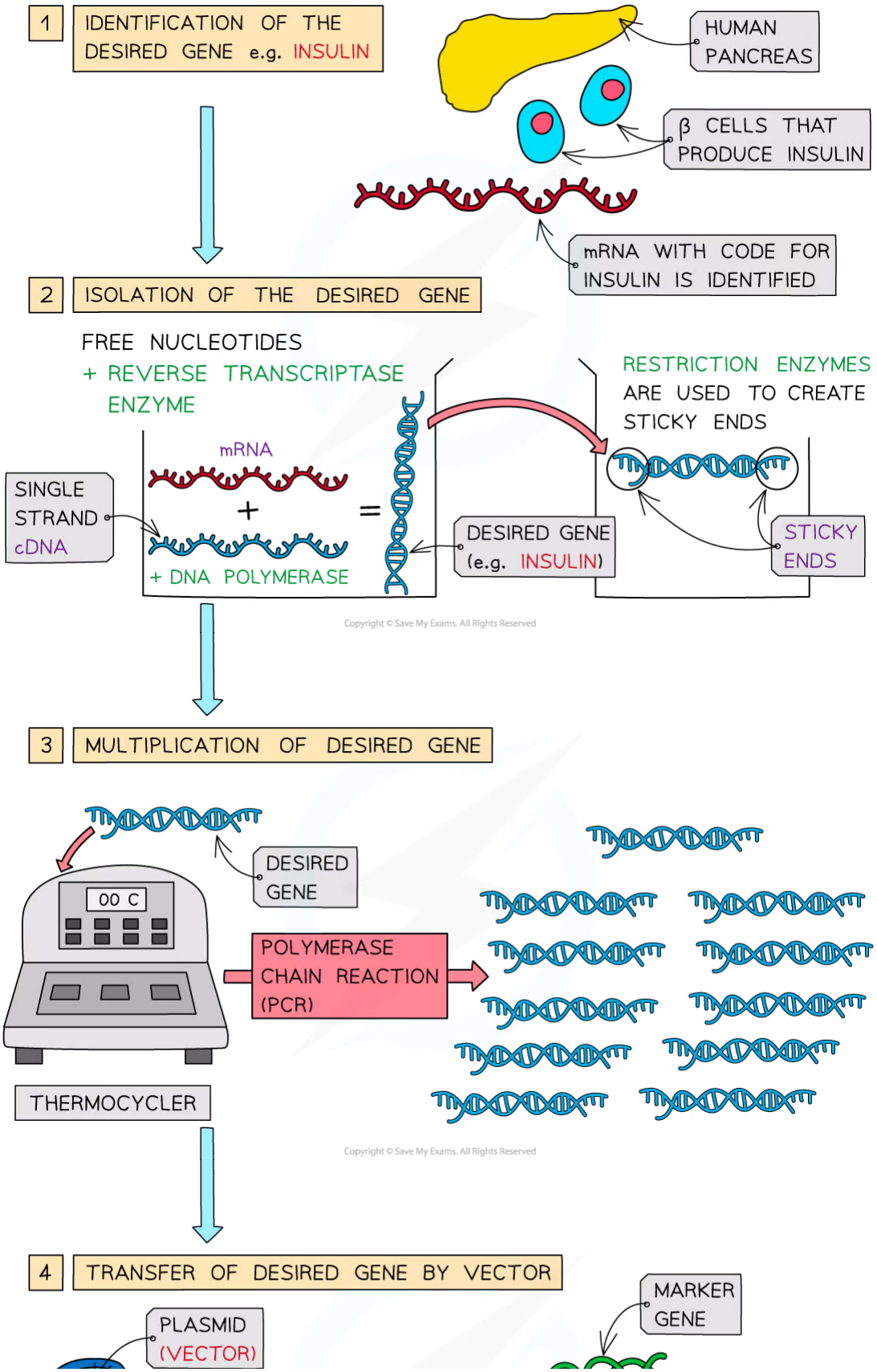
YOUR NOTES

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

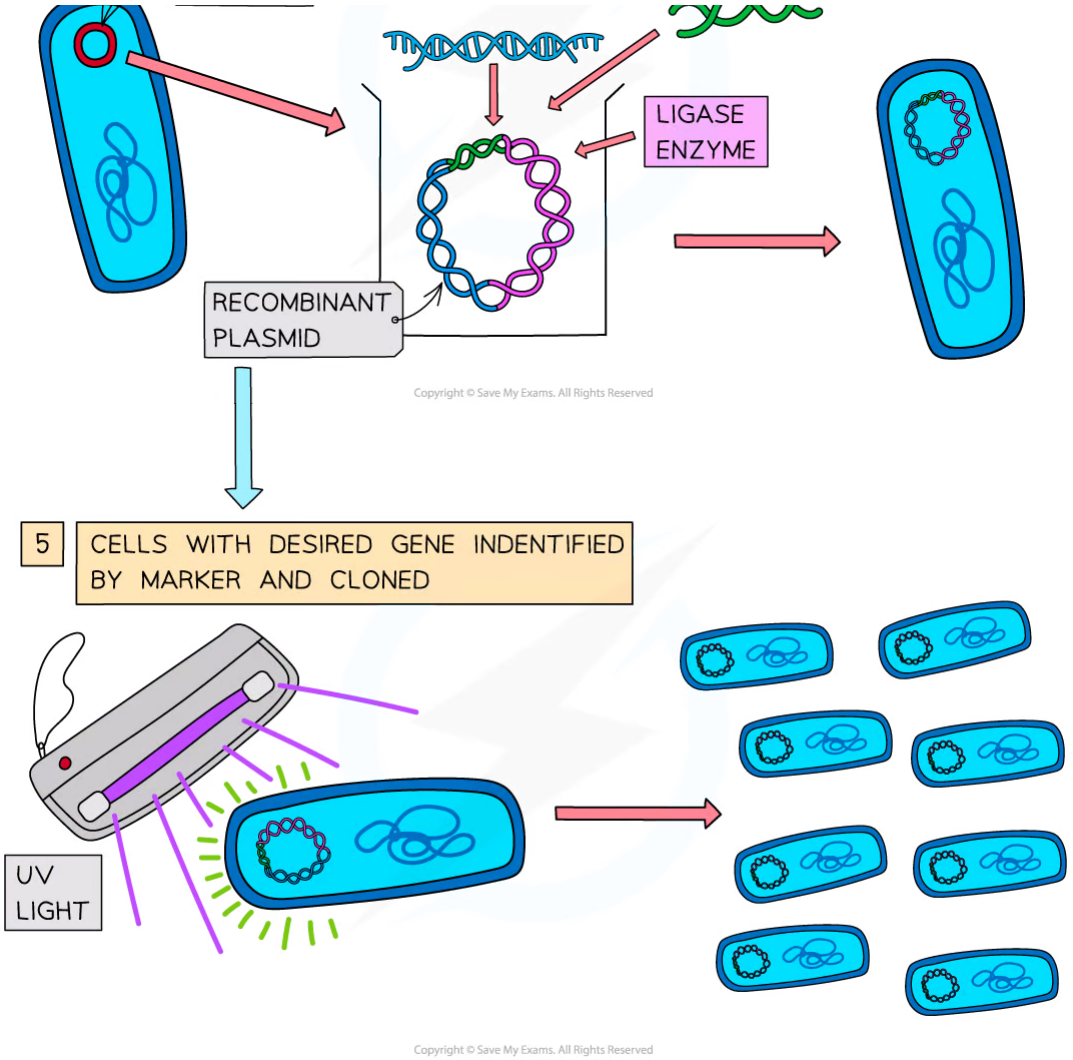
- In order for an organism to be genetically modified the following steps must be taken:
  - **Identification** of the **DNA fragment** or **gene**
  - **Isolation** of the desired DNA fragment (either using restriction endonucleases or reverse transcriptase)
  - **Multiplication** of the DNA fragment (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



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

- 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

YOUR NOTES

- 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

Exam 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

YOUR NOTES

- 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

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

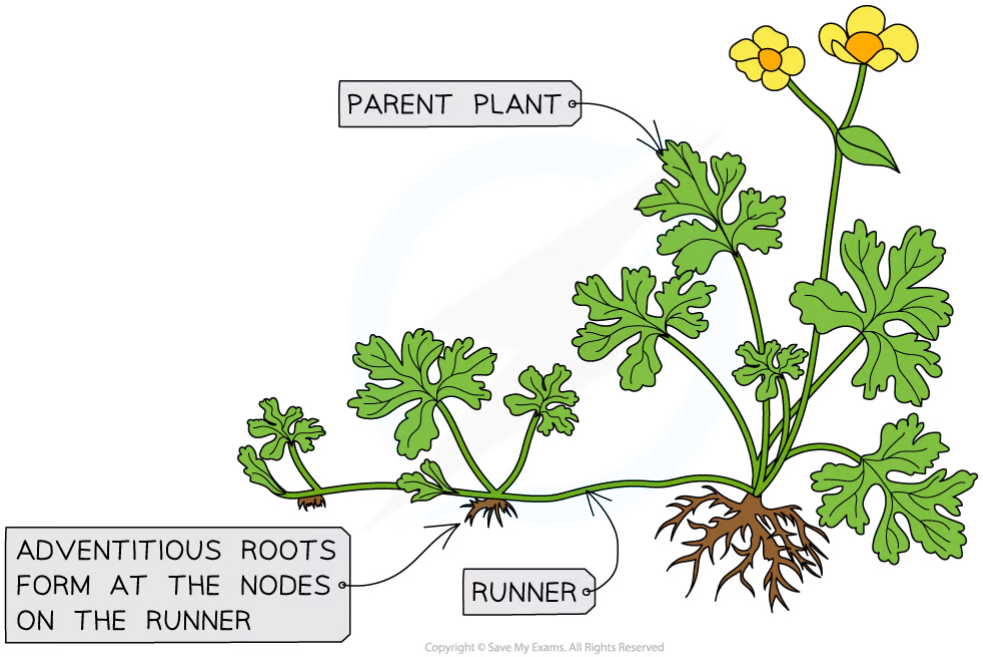
YOUR NOTES

- 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

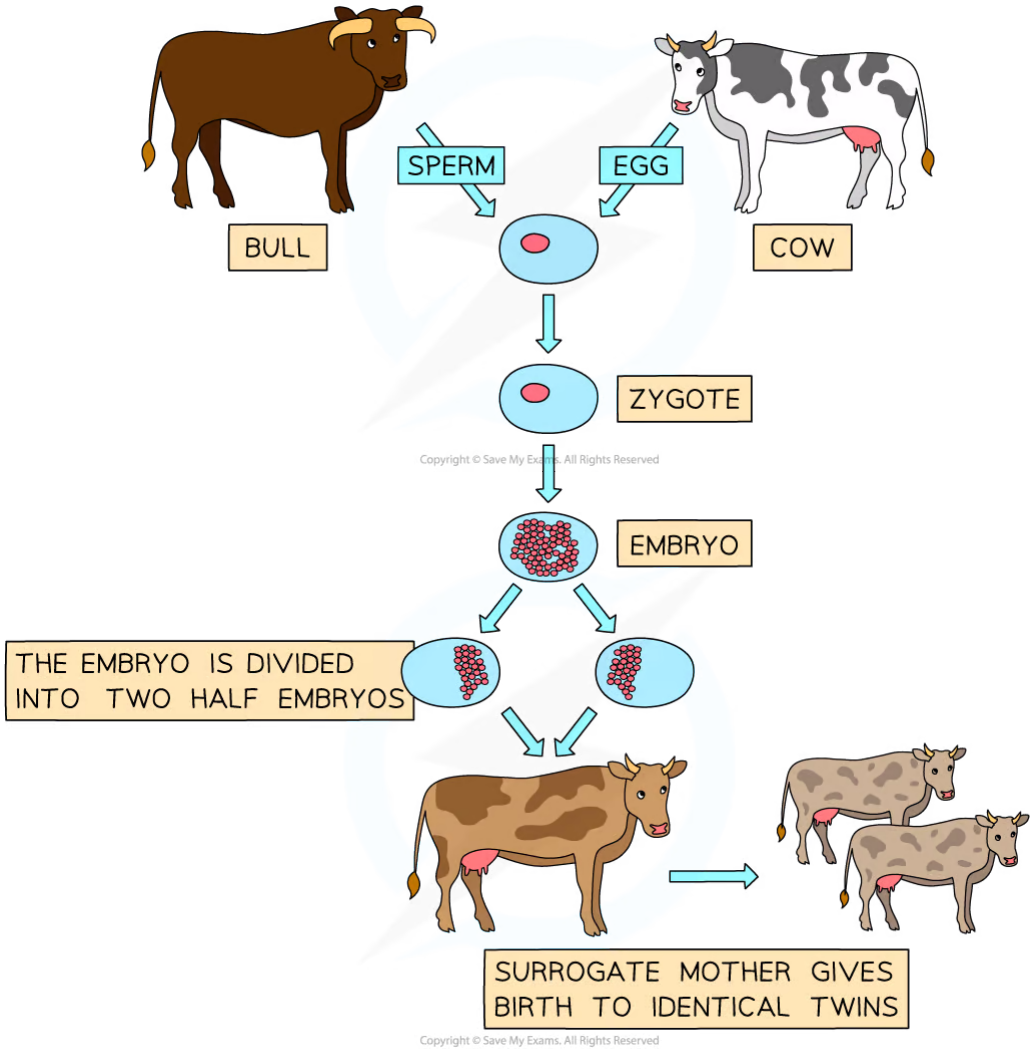
Exam Tip

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

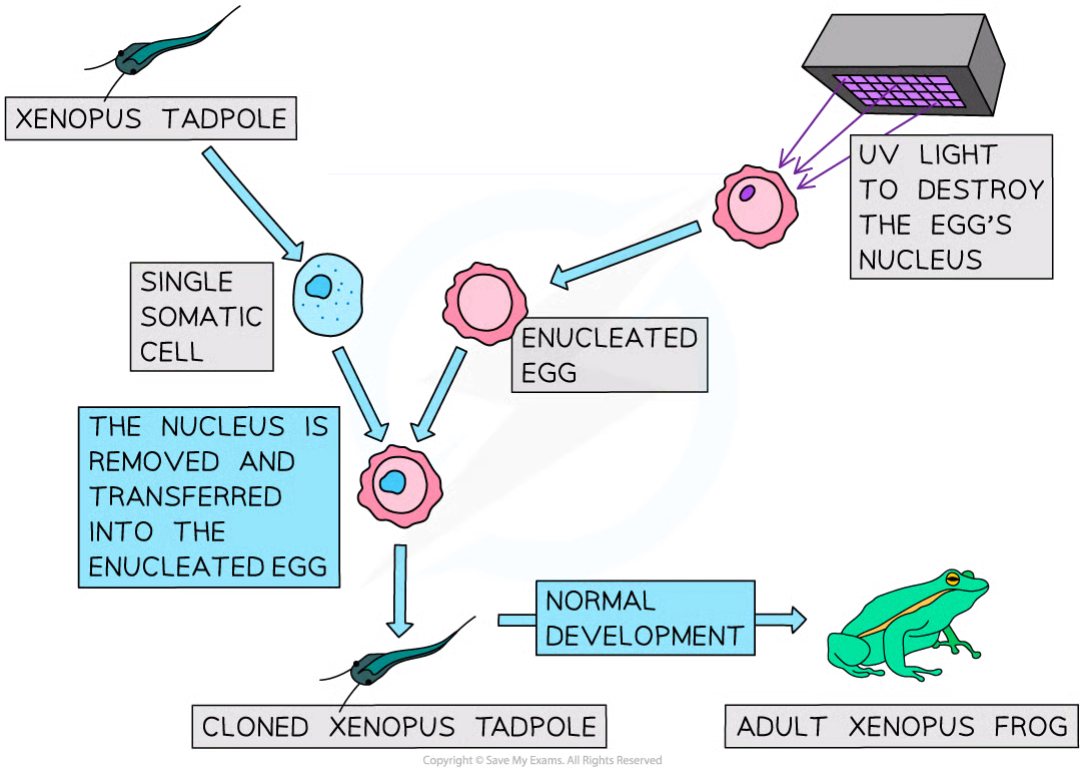
YOUR NOTES

- 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

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



Cloning of Xenopus frogs by nuclear transfer from a differentiated cell

YOUR NOTES

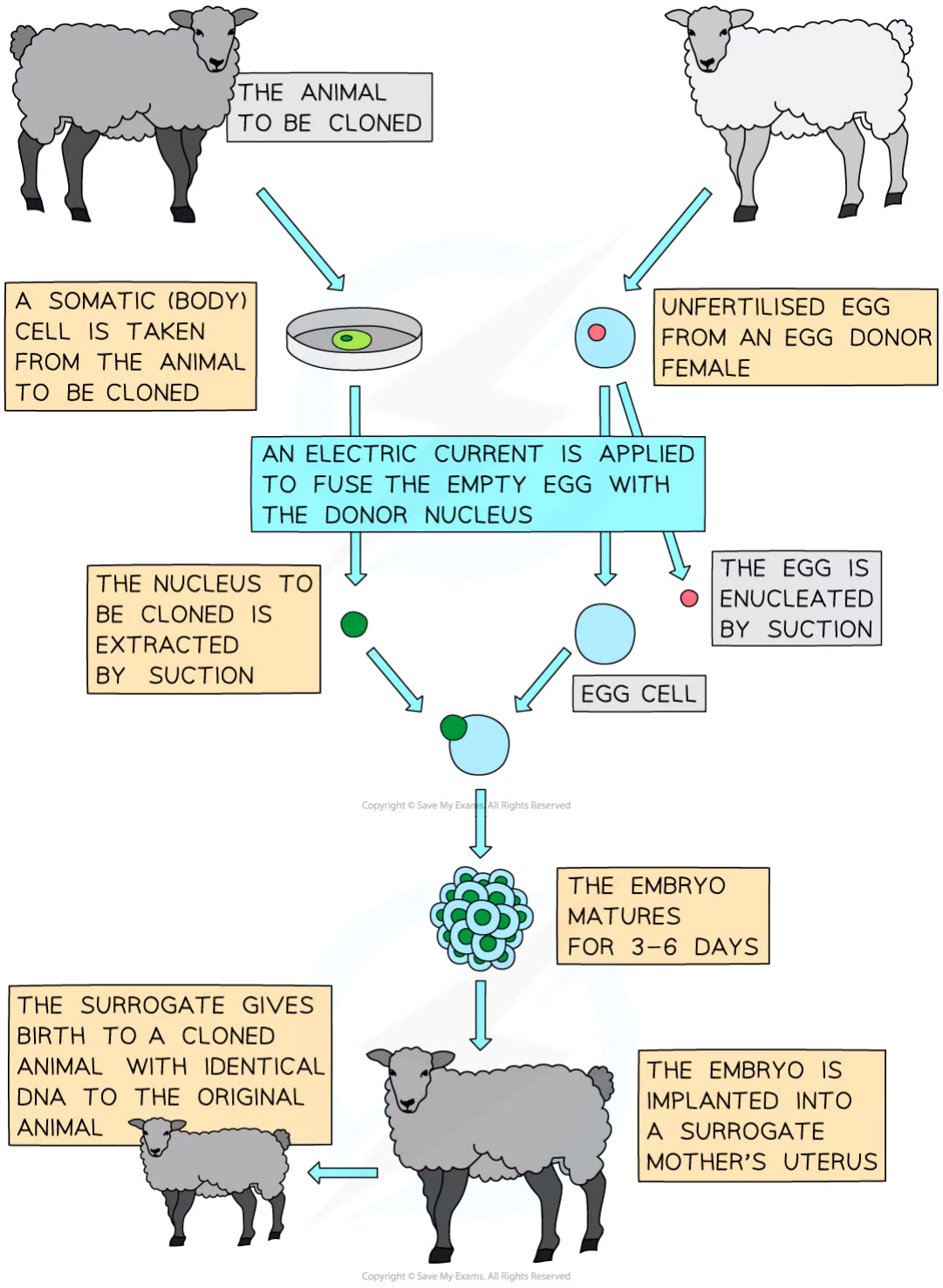
Somatic Cell Transfer

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

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?

YOUR NOTES ⬇

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

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

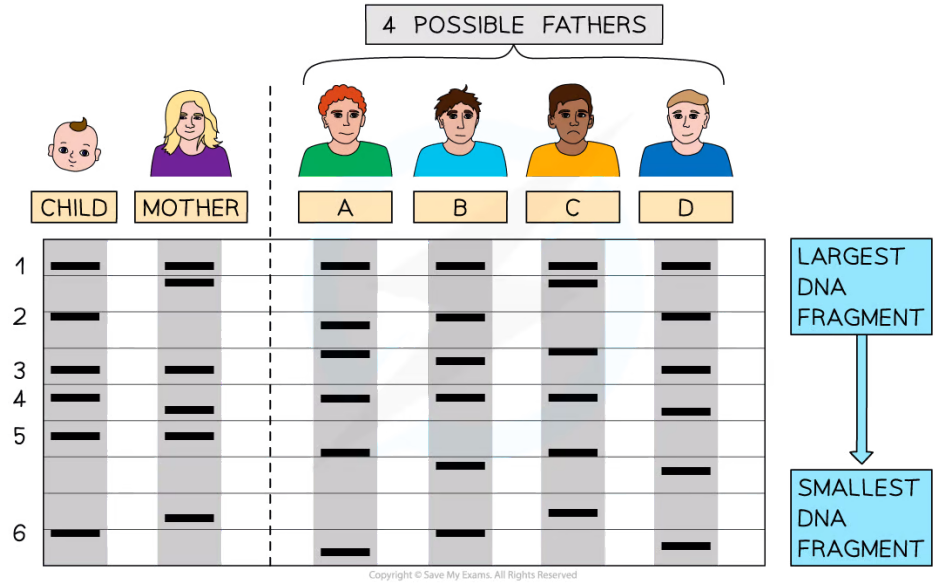
Analysis of DNA Profiles

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

YOUR NOTES

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)

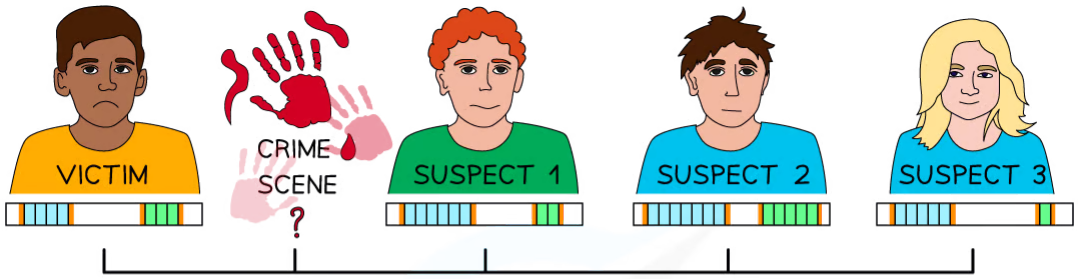
The mother does not possess this band, so the child must have inherited it from its father. Only men A, B and C possess this band, but A and C have already been eliminated

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



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.

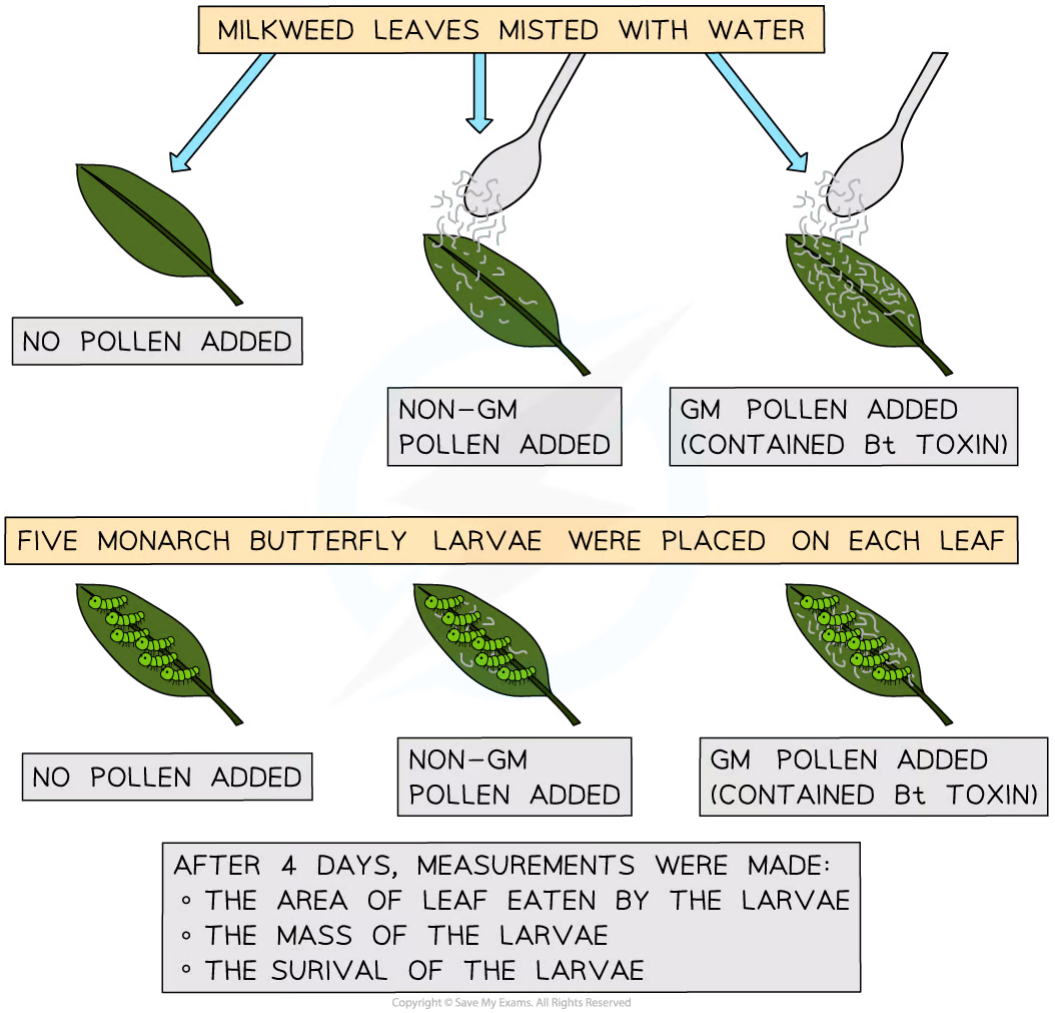
Assessing the Ecological Risks of GM Crops

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

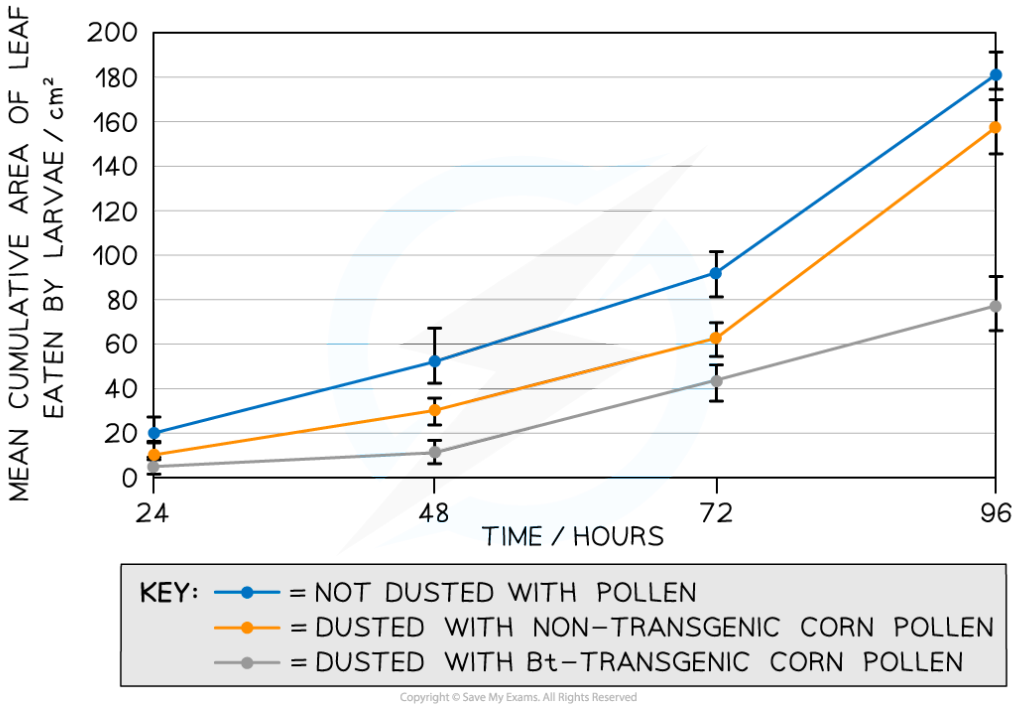


Experimental set-up to prove the effect of Bt toxin-containing pollen on monarch butterfly larvae

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

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						

YOUR NOTES



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

Exam 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