6.4 Cloning & Biotechnology

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6.4.1 NATURAL CLONES IN PLANTS

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Natural Clones in Plants

- Many plants can reproduce asexually using **meristem** cells, in a process called **vegetative reproduction**
- Vegetative organs of plants include:
  - Root and shoot tips
  - Axillary buds (where leaves and the stem meet)
  - Vascular cambium (between xylem and phloem)
- Naturally, over time a young, **miniature plant** (a plantlet) forms at these locations and remains attached to its parent plant
- These plantlets are **clones of their parent** (as no other DNA has been introduced)
- At maturity, the plantlet becomes **detached** from its parent and can live independently, when it is capable of **photosynthesizing** by itself
- The new plants all have the **same phenotype**, so are uniform, making **growing and harvesting easier**
  - Plants that are **hard to grow from seeds** can be propagated, eg. orchids for the horticulture industry
- Some plants have **horizontal stems** or **runners** that form over the soil surface, pointing sufficiently far away so that a new plant at that location will **not be overshadowed** by its parent, or in competition for **water** or **soil nutrients**
  - Roots form under the nodes of runners, called **adventitious roots**
  - The runner dies when the plantlet is self-sustaining
  - **Strawberries, peppermint** and **spider plants** reproduce in this way

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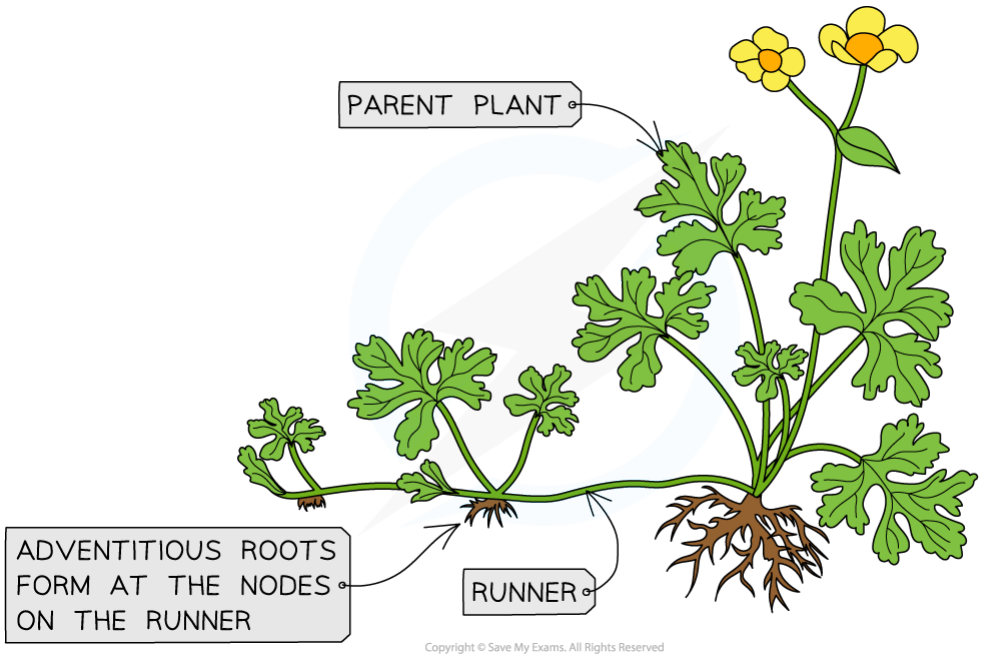

An example of asexual reproduction in plants with runners that form adventitious roots

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Propagation techniques using vegetative reproduction

- Many methods of propagation **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 vegetative propagation contain **modified stems** that can generate **meristematic 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**
- Gardeners and horticulturalists can use these techniques to **propagate desirable species** asexually, effectively and **at less cost** than utilising sexual reproduction techniques
    - This is done by taking cuttings and dividing up plants into different clumps or sections

6.4 Cloning & Biotechnology

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6.4.2 PRODUCING CUTTINGS

Practical: Producing Cuttings

- Many perennials, shrubs, and herbs can grow **adventitious roots** from their cut stems
- The use of cuttings is a straightforward **propagation technique** that can expand plant stock at a very low cost
- **Desirable characteristics** of the plant are **guaranteed** in the progeny because propagation is a form of **asexual** reproduction
- Cuttings can **grow to full-sized plants** much quicker than plants grown from seeds
- Equipment needed:
  - Sharp scissors or secateurs
  - Potting compost
  - Pots to house new plants
  - Hormone rooting powder
  - Plastic bag to cover cutting
- Hormone rooting powder contains **auxins** that promote mitosis and cell **differentiation** in the new root growth
- If the cut is made just below a stem node, **meristem tissue** forms at the lower end of the cut

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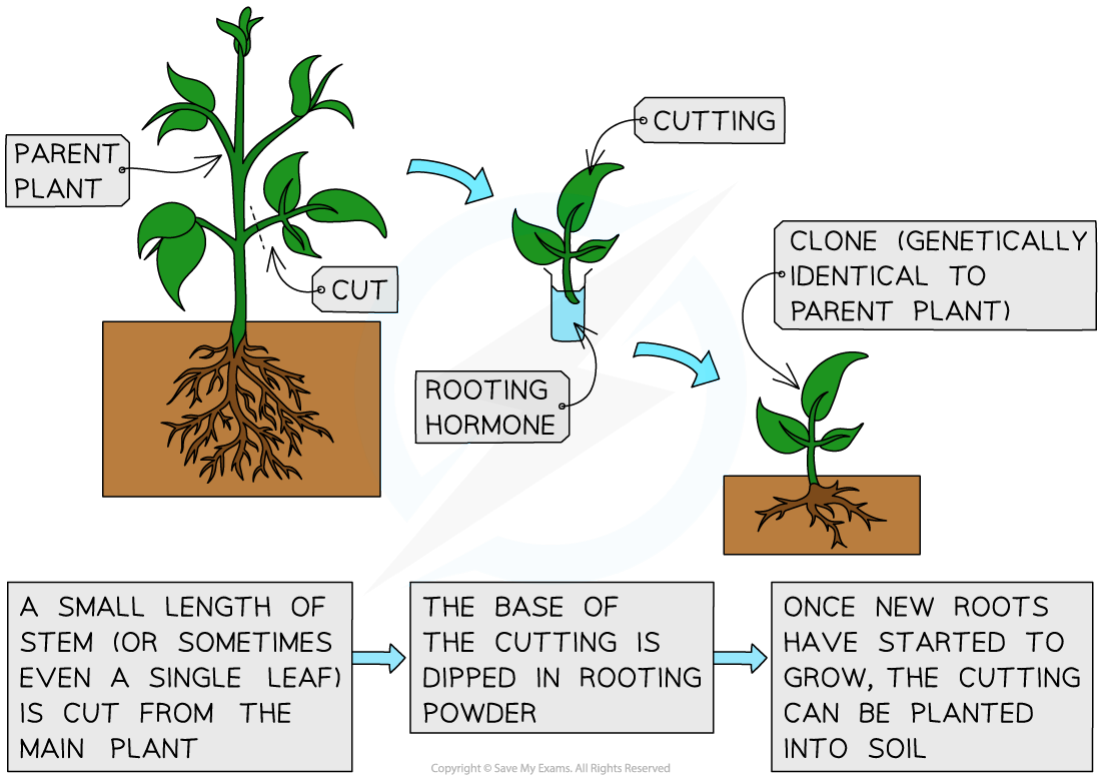

The process of taking cuttings

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6.4 Cloning & Biotechnology

6.4.3 PRODUCTION OF ARTIFICIAL CLONES IN PLANTS

Production of Artificial Clones in Plants

Cultivars

- Gardeners and horticulturalists can **harness the natural processes** of vegetative reproduction to form the basis of artificial cloning
- Techniques such as **cuttings, layering, grafting, division** and **budding** have been used
- This creates **cultivars**, strains of genetically identical plants that can endure as **foodstuffs** or **commercial blooms** for many years
- All methods rely on the formation of **meristematic tissue** from which plant organs can differentiate

Micropropagation and tissue culture

- Many plant cells are **totipotent**, unlike animal cells, and therefore an entire plant can be reproduced from any of these cells
- Creating clones of cauliflowers is used to demonstrate totipotency through the production of **tissue culture**
- Cauliflower is used because it is comprised mostly of **actively dividing cells** and can withstand being handled
- A small piece of the plant is cut, this is called an **explant**, which is then grown into a new clone of the original plant
- Care must be taken to **disinfect the explant** and to use **aseptic techniques** to avoid fungi from colonising the growth medium and causing the micropropagation to fail
- This technique is used by scientists to reproduce **endangered species** of plants where relatively little source material exists

Apparatus

- Eye protection
- Disinfectant (usually a bleach solution)
- Sterilising solution
- Scalpel
- Gloves
- Forceps
- Cauliflower
- Agar growth medium containing sterilant
- Container
- Marker pen

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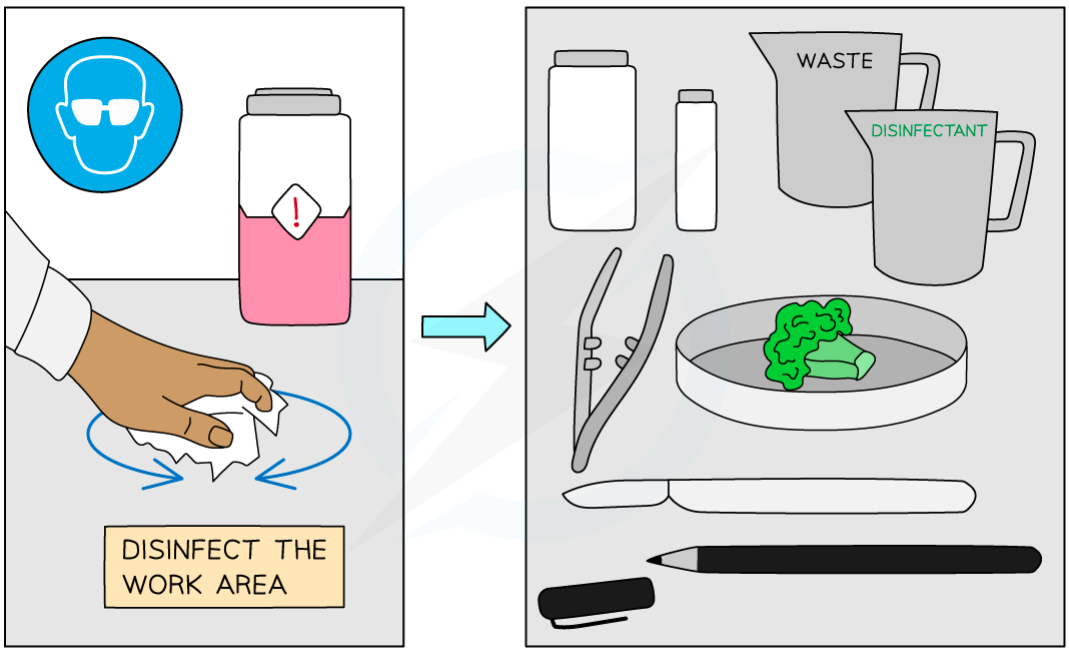
Method

- Wear eye protection at all times
- Wipe all surfaces with disinfectant and soak all apparatus in sterilant
  - It is important to ensure a **sterile environment** so that **no fungi contaminate** the experiment, which would result in seeing a fungal growth rather than an explant growth
- Break off a **small floret of cauliflower** from the plant then using a scalpel, cut a thin section of the floret (about 5-10mm long)
  - This thin section is the **explant**
- Sterilise the explant by soaking it in sterilising solution for 15 minutes, swirling the explant around within the solution every 5 minutes
  - This ensures that the explant is **sterile** and therefore only cauliflower cells are present
- Take out the explant using sterilised forceps and add it to a container of agar growth medium
  - The growth medium contains all the **nutrients that the plant needs for growth** and also contains a sterilant to ensure no contamination occurs throughout the experiment
- Leave the container holding the agar growth medium and the explant on a sunny windowsill for 3 weeks



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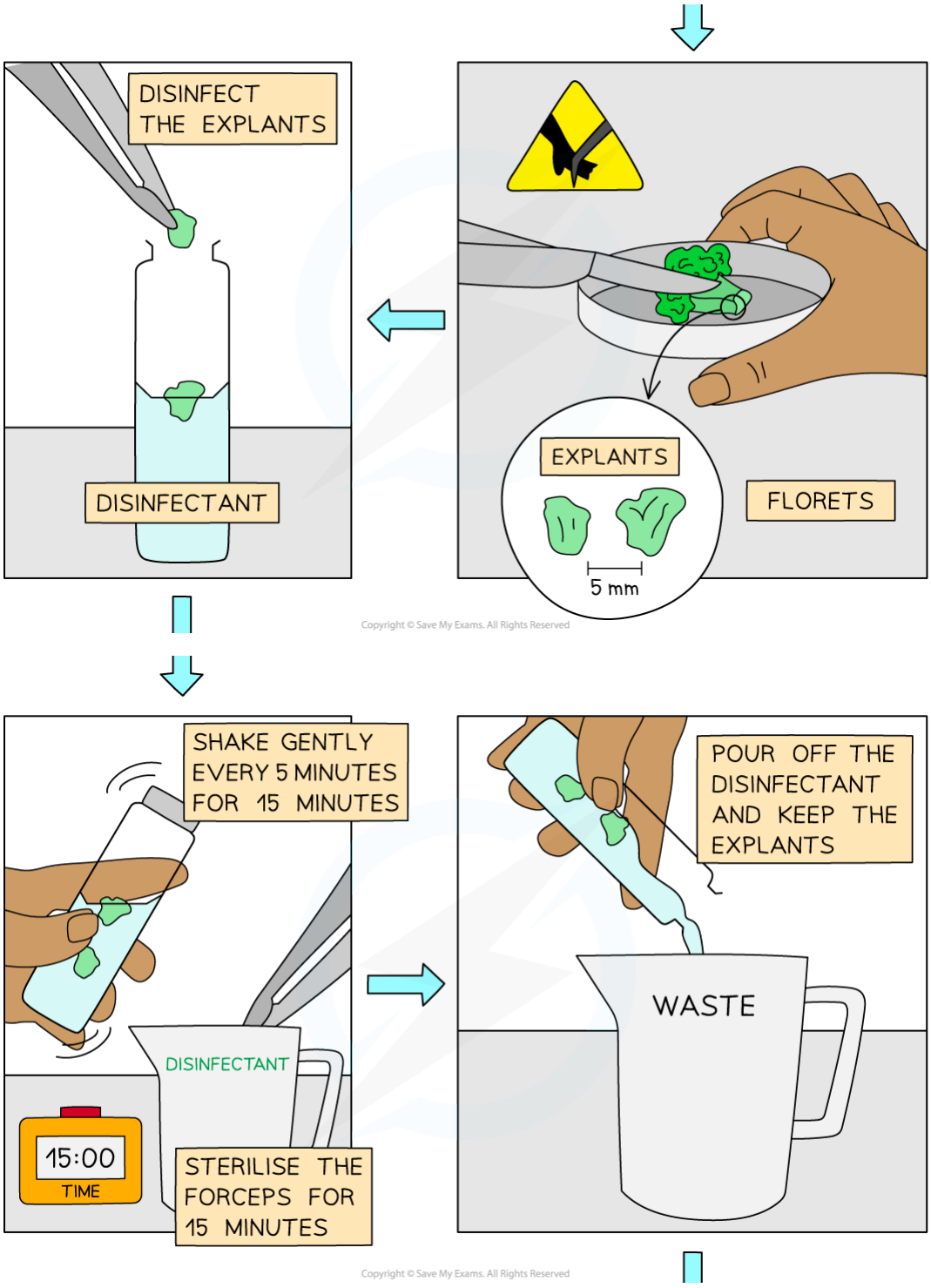

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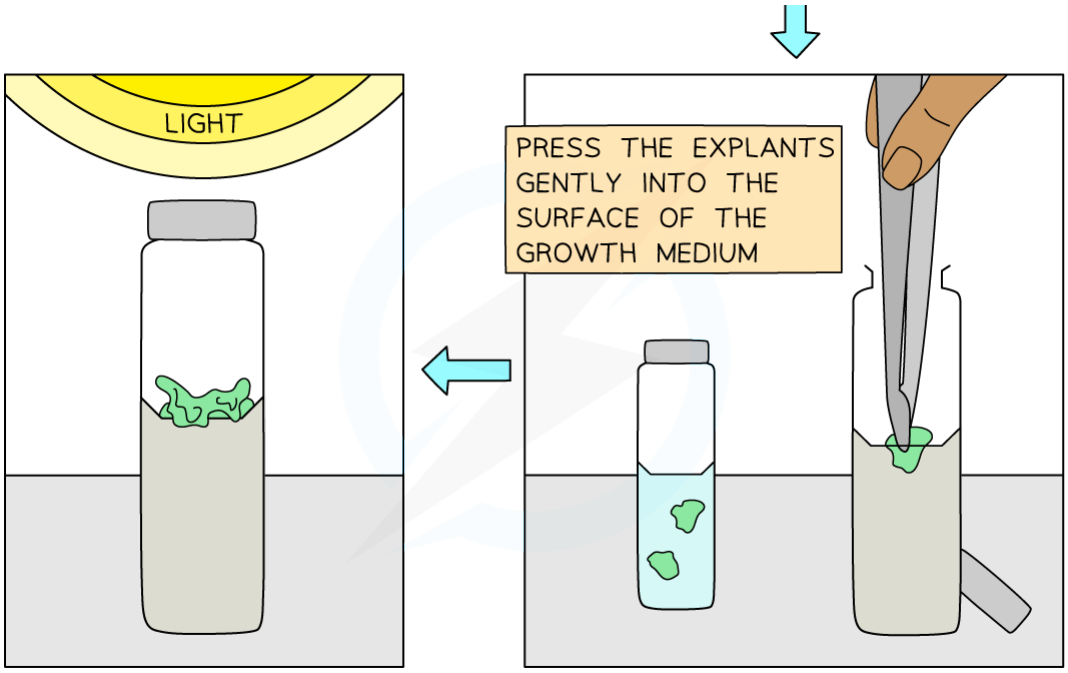

The steps of micropropagation of a cauliflower from an explant

Results

- The result of this experiment is to **grow a complete cauliflower clone** from an explant
- This shows that the cells in the explant have **the capability to produce all the different cell types** that make up a full cauliflower plant, hence they are **totipotent**
- The complete cauliflower plant can then be **distributed to commercial** growers/garden centres **in large numbers**

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

6.4.4 USES OF PLANT CLONING

Uses of Plant Cloning

Advantages of plant cloning

- All the plants have the **same genotype** and hence **phenotype**
- The plants produced are **free of disease**
- The plants can be **genetically modified** to confer **immunity** to certain diseases
    - Genetic modification enables growers to ensure that plants **carry very distinct characteristics**
- The process is rapid and can yield **large numbers of new plants**
- The small plants that are produced can be **transported easily** to other sites
- Plants that are **difficult to grow** from their seeds can be produced by plant cloning
- Plants can be grown **in any country, in any season**
- **Rare and endangered species** can be propagated to save them from extinction
- Whole plants can be created **from genetically modified cells/tissues**
- The use of cultivars **prevents the risk of F_1 hybrids** that occur when plants are crossed and grown from seeds, so the products are **more uniform**

Disadvantages of plant cloning

- It is an **expensive** and **labour-intensive** process
- The process is susceptible to **microbial contamination**
- There is **no genetic variation**, so all of the offspring are susceptible to the same diseases or other environmental factors
    - This **risks large-scale loss** of a country's / continent's crop of a particular plant, so a range of cultivars is recommended
- New plants have to be **carefully screened** for abnormalities that could lead to the new plants being infected
- There is a risk of an **unexpected secondary metabolic chemical reaction** that could cause **stunted growth** or even **death** in the new explants

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

6.4.5 NATURAL CLONES IN ANIMALS

Natural Clones in Animals

Asexual reproduction in animals

- 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**

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
- Two embryos that form are **identical**, with the same genotype and develop *in utero* together
- The result is the birth of **identical offspring**, always of the same gender, with identical phenotype
- Because non-identical twins are formed from separate eggs and sperm, they are **not considered clones**

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.

6.4 Cloning & Biotechnology

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6.4.6 PRODUCTION OF ARTIFICIAL CLONES IN ANIMALS

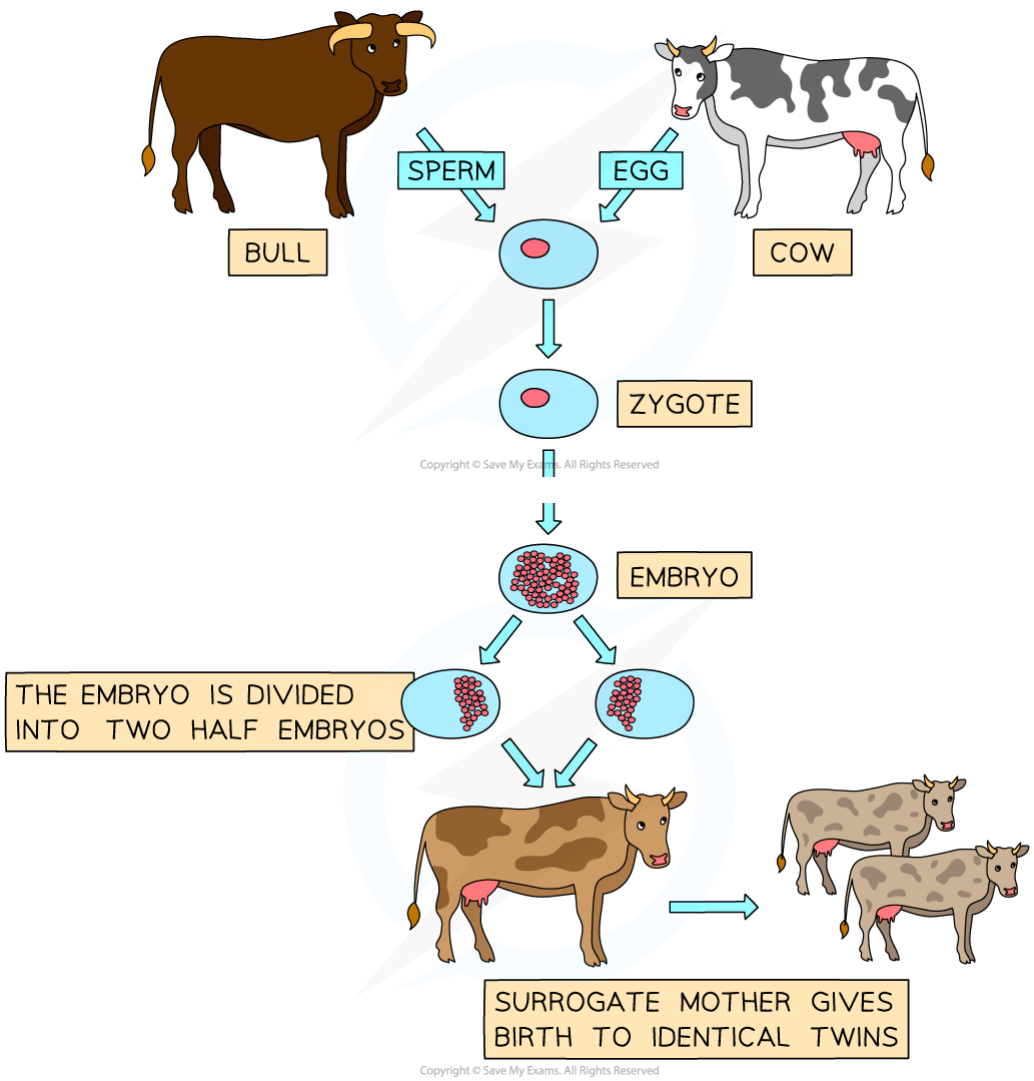
Production of Artificial Clones in Animals

Embryo twinning

- The process of embryo twinning 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
- 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

6.4 Cloning & Biotechnology

YOUR NOTES



Embryo twinning of cattle by splitting the embryo

6.4 Cloning & Biotechnology

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Reproductive cloning

- This is the method made famous by **Dolly the sheep**, cloned in Edinburgh, UK in 1996
- Its 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 to be 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** eg. from an udder
  - 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

6.4 Cloning & Biotechnology

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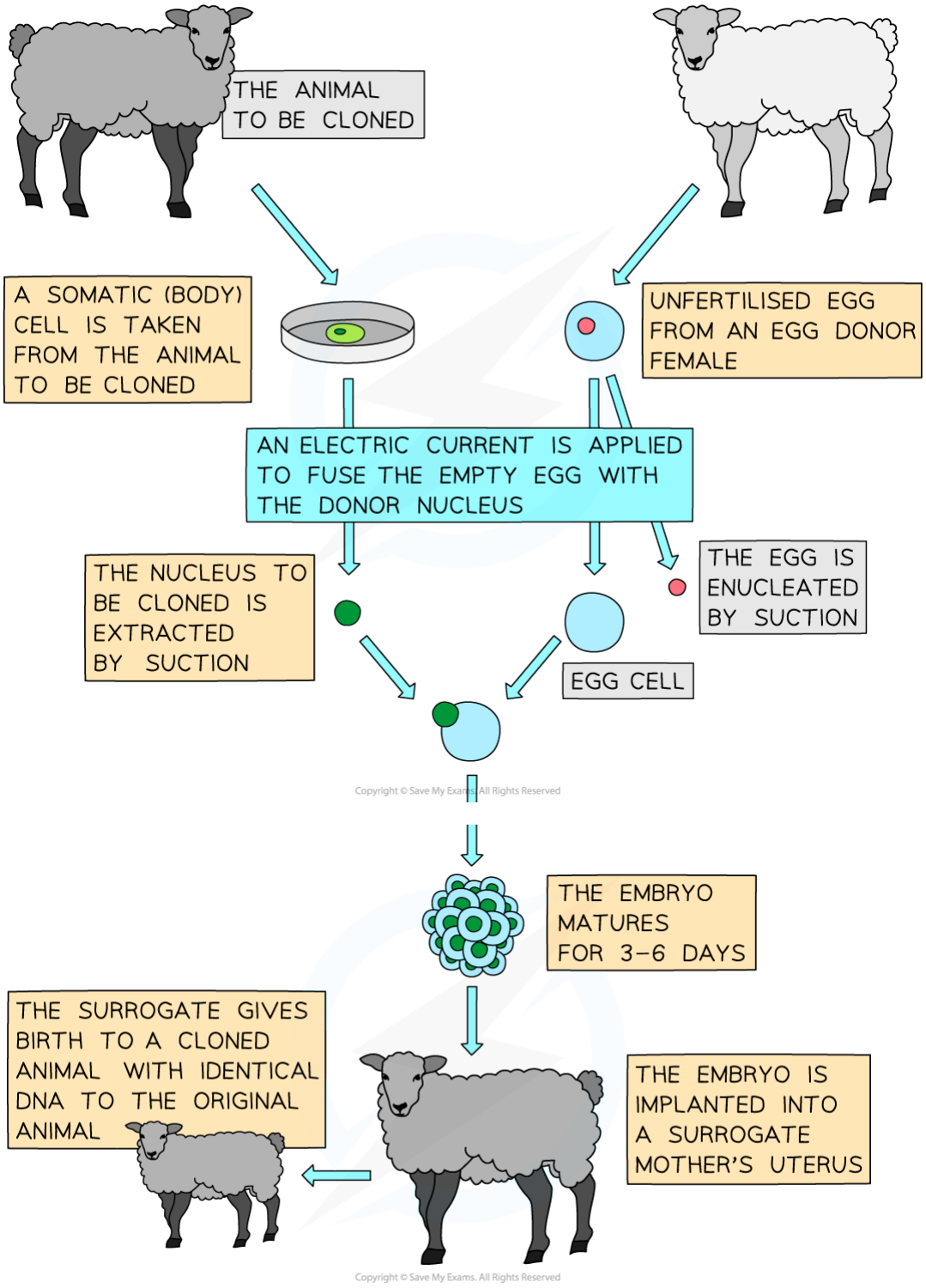

Reproductive cloning of animals.

6.4 Cloning & Biotechnology

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Therapeutic cloning

- This is a technique designed to use cloned cells to **replace dead or damaged cells** that cause a loss of function in an individual
- Embryos are **cloned as in reproductive cloning,** but the embryos are removed and subdivided
- Each individual embryo cell is a totipotent stem cell that 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

6.4 Cloning & Biotechnology

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6.4.7 USES OF ANIMAL CLONING

Uses of Animal Cloning

Arguments for animal cloning

- Embryo cloning is **well accepted** and **noncontroversial** in the field of livestock farming
- Many animals with desirable characteristics can be cloned, ideal for **maximising agricultural output** eg. milk yield in cattle
- Cloning can also:
  - **Remove less desirable characteristics** from the gene pool over time, much in the way that selective breeding has done
  - Help **preserve endangered species**, ahead of possible reintroduction of those species to the wild
  - Provide **regenerated organs** for patients suffering from degenerative disease. Such organs will be a **direct genome match** to the patient so would have **no risk of rejection** by the immune system

Arguments against animal cloning

- The process of **somatic cell nuclear transfer** (SCNT) is very **hit-and-miss**
  - It took hundreds of unsuccessful attempts to clone Dolly the sheep
- There are also **unknown long-term effects** of the cloning process
  - Subsequent cloning attempts have led to a **high number of early deaths** and **genetic abnormalities** in the clones
- Some cloned animals that survive birth and infancy tend to grow **abnormally large** (Large Organ Syndrone – LOS)
  - LOS can cause **breathing and circulatory problems** in adult animals
  - Other clones have developed **abnormalities** in other large organs eg. kidneys, or in their **immune systems**
- No precise 'cause and effect' has been ascribed to the cloning process, but research has indicated that cloning **disrupts the normal mechanisms or regulation of gene expression**
- Cloning **destroys embryos** which could in theory develop into a healthy adult animal – the argument put forward by groups such as Pro-Life
- For these reasons, **animal cloning has not gathered as much pace** as many scientists thought it would

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

6.4.8 MICROORGANISMS & BIOTECHNOLOGY

Microorganisms & Biotechnology

- Biotechnology harnesses the processes in living organisms to
  - Produce useful products, such as **foods and medicines**
  - Carry out **useful services**, such as **sewage treatment, composting** and **bioremediation**
- Microorganisms are the **most useful group of organisms** that carry out biotechnological processes because they
  - Have **simple growth requirements**
    - Their food is **cheap** and **readily available**
    - They occupy **very little space**
  - Reproduce **quickly**
  - Do not have non-productive tissues and organs
  - Can be grown on an **industrial scale** to perform duties useful to large numbers of the human population

Common processes that use biotechnology

Brewing and distilling

- Brewing uses **yeast** species, eg. *Saccharomyces cerevisiae, Saccharomyces pastorianus* to respire sugars from **barley malt** and produce **ethanol** and **CO_2**
- The process is anaerobic, and is known as **fermentation**
- **Ethanol** is the primary product, and **CO_2** is a by-product
- Some alcoholic drinks have a higher alcohol content than fermentation alone can produce, so these drinks are first fermented, then **distilled** to concentrate the alcohol
  - **Whisky** and **bourbon** are distilled from a barley beer
  - **Brandy** is distilled from grape wine
  - **Other spirits** use generic fermented ethanol distilled through **botanicals** (berries, herbs and spices) to **extract flavours**
    - E.g. Gin, which uses mainly juniper berries

6.4 Cloning & Biotechnology

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Baking bread

- Wheat or rye flour is mixed with **yeast** and other ingredients to make **dough**
- The culture of yeast is in fact a **mixture of several different naturally occurring species**
- Commercial bakeries **carefully control the species** of yeast used
- Artisan bakeries sometimes use **wild yeast cultures,** preserved and cultivated regularly (sometimes called 'starter cultures')
- Yeast **enzymes** begin by **hydrolysing the starch** in flour to **maltose**
- **Maltose** is then hydrolysed to produce **monosaccharides** which can be used for **aerobic respiration**
- When oxygen runs out, yeast begin to respire **anaerobically**
- Both aerobic and anaerobic respiration produce **CO_2 in bubbles** throughout the dough, causing the dough to **rise**
- **Baking kills the yeast** and causes the gas pockets in the dough to expand, so the bread rises further

Cheesemaking

- **Pasteurised milk** is used as a raw material
- **Bacteria** are used to digest lactose, producing **lactic acid**
- Lactic acid **lowers the pH** of the milk
- The low pH causes proteins in the milk to denature, leading to separation of **curds** (solids) and **whey** (liquids)
- Curds are **pressed and processed** into hard cheeses eg. Red Leicester
- **Mould spores** from saprotrophic fungi such as *Penicillium glaucum* can be artificially introduced into **blue-veined cheeses** eg. Gorgonzola

Yoghurt making

- A **starter culture** of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* **bacteria** are introduced to **pasteurised** milk
- The bacteria use sugars in the milk to respire and produce lactic acid as a waste product
- Lactic acid **denatures** the proteins in the milk, causing them to **coagulate** (stick together). This produces the thick texture and and **sour taste** of yoghurt
- Flavours can be added at this stage to produce flavoured yoghurt

6.4 Cloning & Biotechnology

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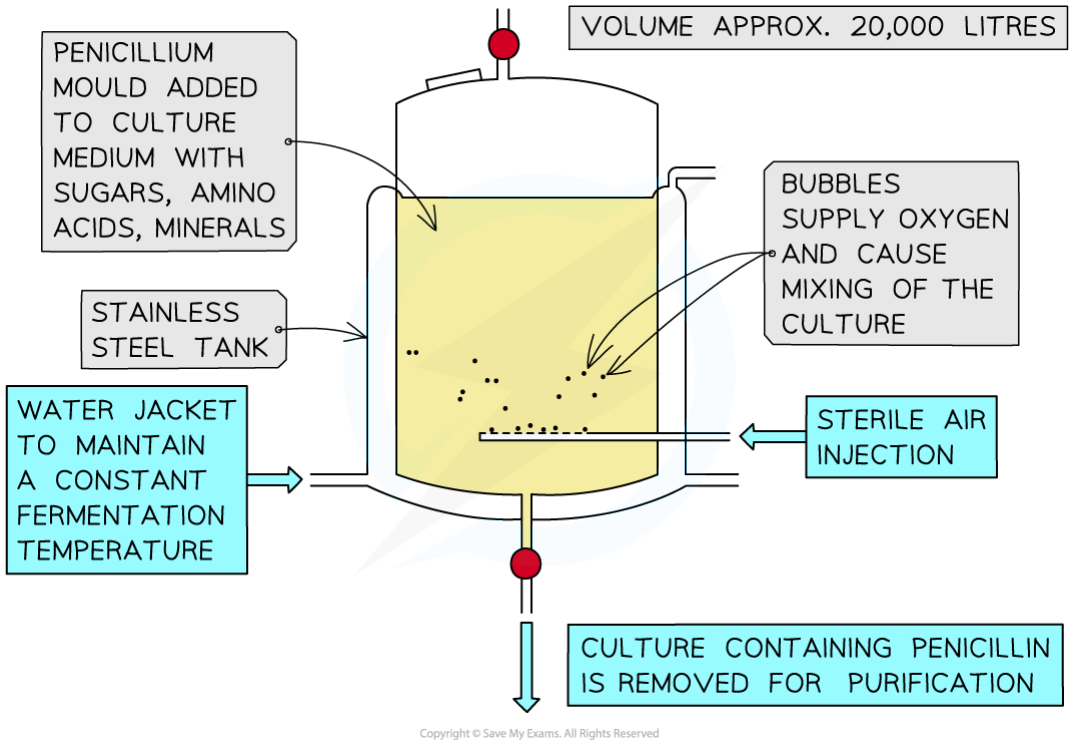
Penicillin production

- Species of mould from the ***Penicillium*** genus can be cultured in **industrial fermenters**
- The technique is known as deep-tank fermentation
- **Extraction** and **purification** of the product produces large volumes of the drug for therapeutic use
- Penicillin became one of the first 'wonder drugs' as a result of being produced on a large scale



A batch fermenter for the commercial production of penicillin

Insulin production

- Large scale production of **human insulin** can be carried out using biotechnology
- Previously, diabetics had to be treated with pig insulin, which is **hard to isolate**, **expensive** and **not as effective** as human insulin
- **Recombinant DNA technology** can incorporate the gene for human insulin into the genome of the bacterium, *Escheriscia coli*
- Recombinant bacteria are grown in **batch fermenters**, and each bacterial cell **expresses insulin**
- Insulin is **released into the batch medium** and can be **purified** for medicinal use at a later stage

6.4 Cloning & Biotechnology

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Mycoprotein production

- Mycoprotein is a **meat substitute** product used to make **vegetarian**, meat-like products like burgers and sausages
- Mycoprotein is **low-fat** and **high in fibre**, so making it an attractive alternative to meat
    - This could play a part in ensuring that a growing human population **eats enough protein**
- The prefix 'myco' means **fungus**
- The microorganism used is *Fusarium venenatum*, a **filamentous fungus**
- A source of **glucose** is added to the tank
- **Oxygen** is also supplied to ensure **aerobic respiration** can occur, which yields maximal growth of hyphae (the part that forms the meat-like material)
- **Nitrogen** is introduced in the form of ammonia
- Quorn™ is a well-known **brand name** for mycoprotein
- The product in mycoprotein is the **fungus itself**, rather than a substance produced by microorganisms

Bioremediation

- Humans can contaminate land and water with **toxic substances** through their activity
- Remediating this land can **remove the pollutants** and restore the land to its natural state
- Examples are **oil spills, industrial accidents, acidic damage** from mining and **cleanup of crime scenes**
- Many bioremediation techniques rely on **oxidative digestion** of pollutants
- Bioventing is sometimes all that is needed, a process which allows oxygen to reach the contaminants
    - Naturally occurring microorganisms perform **aerobic digestion** of the contaminants and release **non-polluting products**
- Biostimulation also relies on naturally occurring microorganisms but **adds nutrients** that promote microbial digestion of pollutants
- **Genetic engineering** has been trialled to create microorganisms that are capable of bioremediation

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Emerging uses of biotechnology

- As well as the established processes listed above, research on biotechnology has identified possible roles in these emerging processes
  - Production of **biofuels** (to replace the use of fossil fuels)
  - Production of **vaccines** and **antibodies** for the treatment of disease
  - Production of **hardy crop plants** that can grow in arid conditions
  - Counteracting threats from **bioterrorism** or **bio-warfare**
- All of these potential applications are in their infancy but are in research and development trials

6.4 Cloning & Biotechnology

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6.4.9 MICROORGANISMS & FOOD PRODUCTION

Microorganisms & Food Production

The Use of Microorganisms for Making Food

- There are compelling reasons for using biotechnology to make **human food** and **animal feed**
- The **human population is growing** quickly, so there is a need for ever-more **productive** methods of food production
- There are **advantages** and **disadvantages** of using microorganisms for making food

Advantages and Disadvantages of Using microorganisms to Make Food Table

Advantages	Disadvantages
Animals are less efficient than microorganisms at converting energy into biomass	People may not like the thought that their food has been produced by microorganisms, so may not buy the product
Microorganisms reproduce more quickly than plants and animals, so selective breeding is quicker	A fermenter contaminated by other bacteria could lead to a ruined product
Microorganisms can be grown on substrates that are waste products of other industries, such as whey, a waste product from cheese-making	Sterile technique is important to avoid contamination, but is expensive

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Growth is not seasonal	Microorganisms reproduce quickly, therefore mutate quickly, so undesirable strains may arise
Inputs are cheap eg., glucose, oxygen, ammonia, and large amounts of land are not required	Fermenter-produced products are often high in nucleic acids, which can be harmful to humans when consumed in large quantities. Processing is therefore required to ensure that food is safe to eat
Fermenters can be set up anywhere in the world, so food can be produced in places where crops and livestock cannot survive e.g. very hot or cold climates	The perception that the food is nutritious but has little or no flavour/pleasant texture
Low-fat, high protein products eg. mycoprotein	Bacteria can be infected by viruses, which could ruin a product

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6.4.10 CULTURING MICROORGANISMS

Culturing Microorganisms

- When investigating the effect of antimicrobial substances on microbial growth it is essential that **aseptic techniques** are used
- Aseptic techniques ensure the **microbes being investigated don't escape** or **become contaminated** with another unwanted, and possibly pathogenic, microbe
- Some general aseptic techniques include:
  - **Washing hands** thoroughly
  - **No food or drink** allowed in the lab
  - **Disinfecting** work surfaces with disinfectant or alcohol
  - Wearing **gloves** and **goggles**
  - Working close to a **lit Bunsen burner** (this creates an updraught of air so prevents contamination from airborne fungal spores, for example)
  - Flaming equipment (to kill microorganisms or create updraughts)
  - Sterilising (in an **autoclave**) or disposing of all used equipment

Culturing method

1. Pour the sterile agar into the petri dish, cover with the lid and leave to cool
2. Sterilise the inoculating loop in the Bunsen burner flame
3. Remove the plug and flame the neck of the culture tube
4. Take a sample from the culture tube using the inoculating loop
5. Flame the neck of the culture tube again before replacing the plug
6. Wipe the end of the loop gently on the surface of the agar
7. Sterilise the loop again
8. Tape the lid of the petri dish
9. Incubate at 25°C for 24 hours

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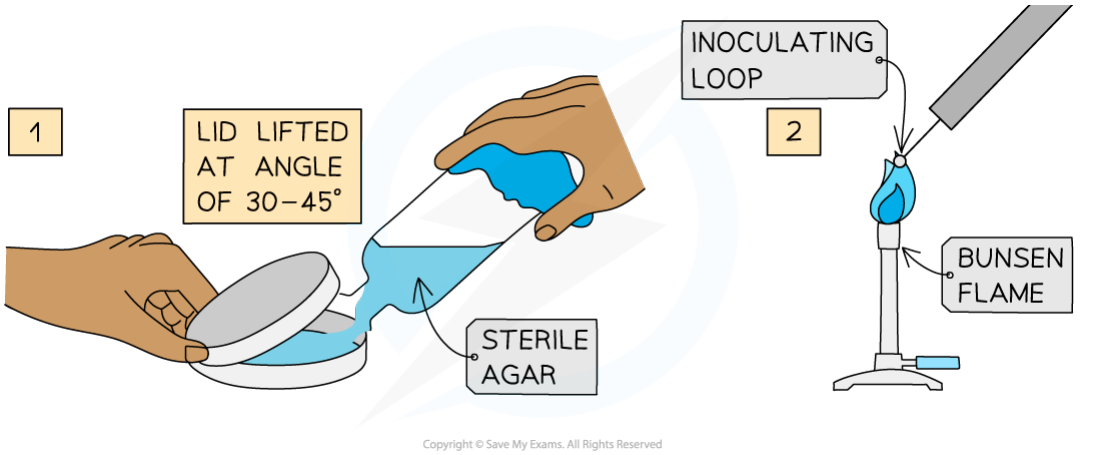
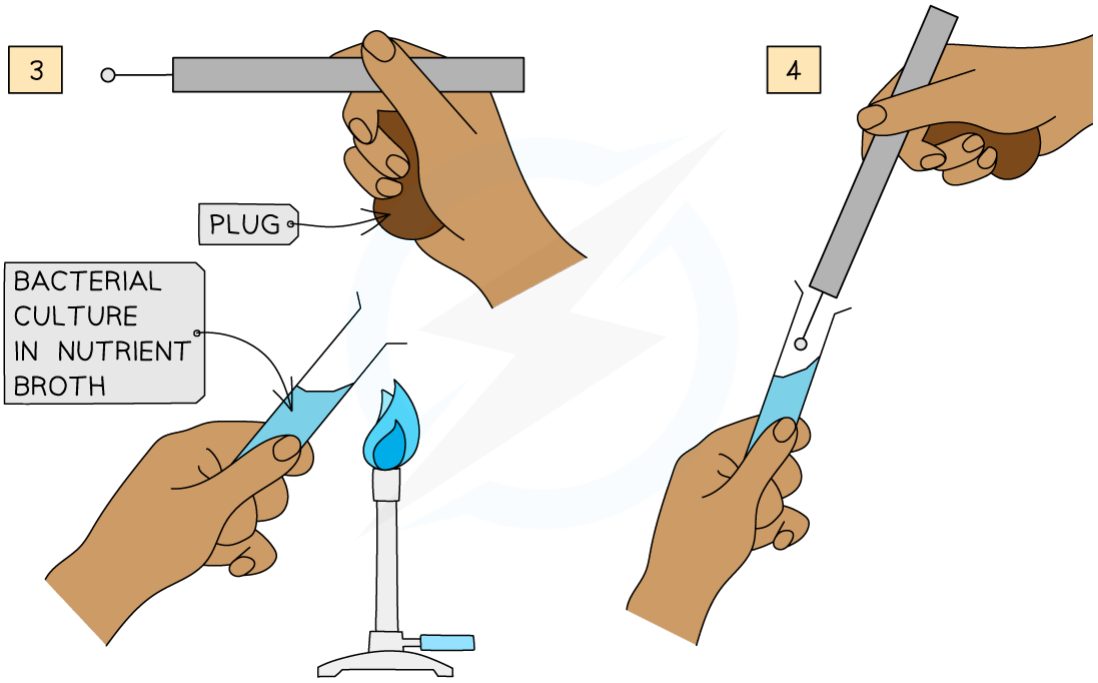
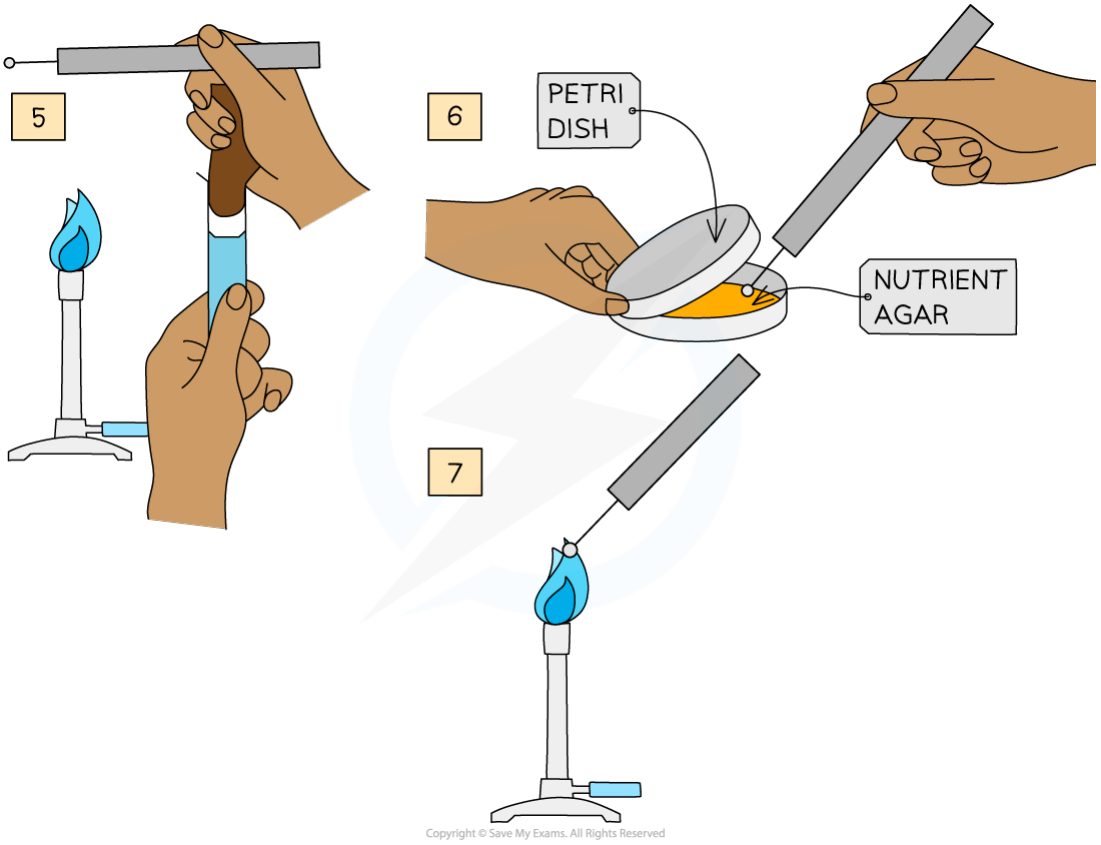
YOUR NOTES





6.4 Cloning & Biotechnology

YOUR NOTES



To prepare an uncontaminated culture of microorganisms, this procedure can be followed

Aseptic Techniques Table

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

STEPS	EXPLANATION
1. WHENEVER WORKING ASEPTICALLY, ALL WORK SHOULD BE CARRIED OUT IN FRONT OF A LIT BUNSEN BURNER WITH A YELLOW FLAME.	THE FLAME CREATES A CONVECTION CURRENT ABOVE THE BENCH, PREVENTING CONTAMINATION OF ANY MICROORGANISMS IN THE AIR.
2. HOT AGAR JELLY IS POURED INTO A STERILISED PETRI DISH. THE AGAR IS LEFT TO COOL AND SET.	THE PETRI DISH AND CULTURE MEDIUM ARE HEATED TO A HIGH TEMPERATURE TO KILL ANY POTENTIAL MICROORGANISMS THAT COULD CONTAMINATE THE EXPERIMENT.
3. AN INOCULATING LOOP IS PASSED THROUGH A HOT FLAME BEFORE IT IS USED TO TRANSFER BACTERIA TO THE CULTURE MEDIUM.	ANY MICROORGANISMS ON THE LOOP ARE KILLED TO PREVENT CONTAMINATION.
4. PETRI DISHES SHOULD ONLY BE OPENED AS LITTLE AS POSSIBLE, AT THE SIDE FACING THE BUNSEN BURNER.	THIS DECREASES THE RISK OF MICROORGANISMS CONTAMI-NATING THE DISH.
5. THE LID OF THE PETRI DISH SHOULD BE SECURED WITH TAPE AT INTERVALS AROUND THE DISH AND STORED UPSIDE DOWN.	THIS PREVENTS DROPS OF CONDENSATION (ANOTHER SOURCE OF CONTAMINATION) FROM DROPPING ONTO THE SURFACE OF THE AGAR.
6. THE CULTURES SHOULD NOT BE INCUBATED ABOVE 25°C IN A SCHOOL LABORATORY.	THIS RESTRICTS THE GROWTH OF HARMFUL PATHOGENS (WHICH ARE MORE LIKELY TO GROW AT HIGHER TEMPERATURES).

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

Investigations using cultured microorganisms

- It is possible to test the efficacy of different **antibiotics** and **antiseptics** using cultured microorganisms
- The **disc diffusion** method is commonly used to test for **antibiotic resistance** in bacteria
  - It allows for **multiple antibiotics** to be tested at once

Apparatus

- Sterile agar plates
  - The agar can be made sterile by boiling
- Diluted bacterial broth with a concentration of 1×10^8 CFU mm^{-3}
  - Colony-forming unit (CFU): a live bacterial cell that is able to divide and form a colony on the agar plate
- Multiple different antibiotic solutions of a standard concentration
- Paper disks
- Pipettes
- Spreaders
- Bunsen burner
- Gloves
- Goggles
- Incubator
- Autoclave

Method

- Pre-soak paper discs in the different antibiotic solutions
  - The different antibiotic solutions need to be the **same concentration** so that the effects of the different antibiotics can be compared
- Spread a sample of the diluted bacterial broth onto the surface of the sterile agar plate
- Lightly press the paper discs onto the surface of the agar
  - Make sure the discs are evenly distributed in the plate
  - They should not be touching the edges of the plate or any other discs
- Keep the agar plate in the incubator overnight
  - The incubator maintains an optimum temperature for bacterial growth
- Remove the agar plate from the incubator and examine the results with the petri dish lid on

6.4 Cloning & Biotechnology

YOUR NOTES

Results

- Overnight the antibiotics will diffuse outwards from each paper disk so that a gradient of antibiotic forms. The antibiotic is most concentrated where the paper disk is located
- If the bacteria being investigated is vulnerable to an antibiotic then a clear area will be visible around the disc
    - There are **no bacteria present in the clear area**
- The clear area will end when the concentration of antibiotic reaches a level that the bacteria are no longer susceptible to
- **More effective antibiotics** require a **lower concentration to kill bacteria** and so they will produce **larger clear zones**
- If a bacteria is **completely resistant** to an antibiotic then there will be **no clear zone** around that particular paper disk

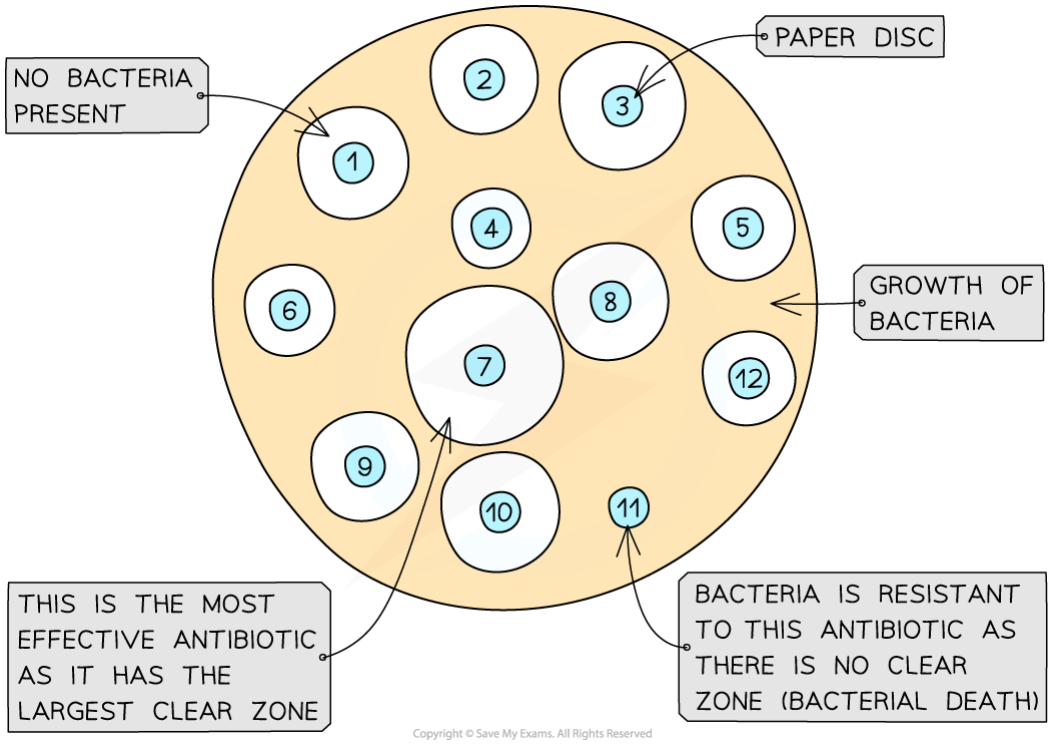
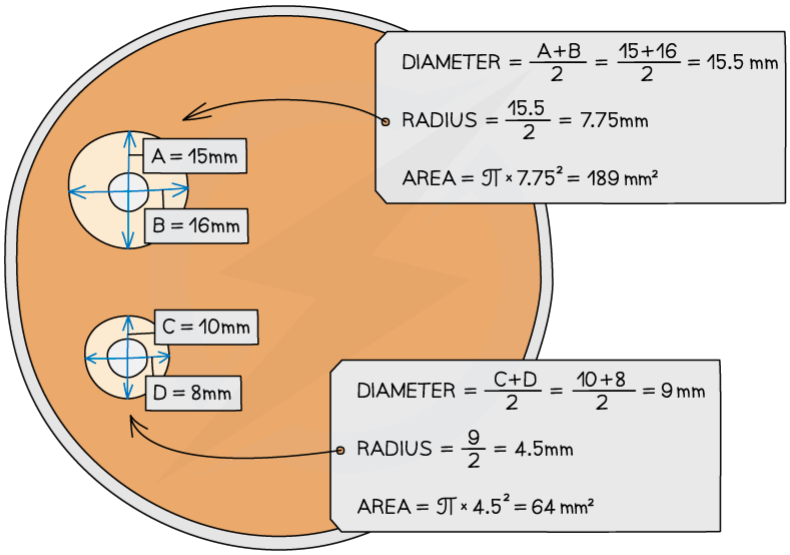


Image showing the bacterial growth on an agar plate following a disc diffusion experiment. The most effective antibiotics produce the largest clear zones while. The antibiotics that the bacteria are resistant to produce no clear zone.

6.4 Cloning & Biotechnology

YOUR NOTES



We can then calculate the area of the zone of inhibition in order to compare the results quantitively

The minimum inhibitory concentration (MIC)

- When antibiotics are used to treat bacterial infections, the dosage used is carefully controlled
- The minimum inhibitory concentration (MIC) is the **lowest concentration of a substance that will inhibit the growth of a microorganism**

Exam Tip

It is expected that you will be able to suggest aseptic techniques that should be used for specific experiments. Make sure to learn a few of the ones above so that you can get those marks!

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

6.4.11 BATCH & CONTINUOUS FERMENTATION

Batch & Continuous Fermentation

- Microorganisms can be grown under **controlled conditions** in larger fermenters
- The product of fermentation is either the **microorganisms itself** e.g. mycoprotein, or a **product** produced by the microorganism e.g. insulin
- There are two main methods used to culture microorganisms by fermentation:
  - **Batch fermentation**
    - Microorganisms are grown in batches in the fermentation vessel
    - Once the culture cycle is complete, the product is removed, the fermenter is cleaned and a new batch of microorganisms is grown
    - This is known as a **closed culture**
  - **Continuous fermentation**
    - Microorganisms are continually grown and the products harvested
    - Nutrients are added and waste is removed throughout the culturing process

Maintaining optimum conditions in the fermenter

- In order to maximise growth and productivity of the microorganisms, the fermenter must be carefully monitored and controlled to ensure conditions are optimum

Optimum Conditions for Fermentation Table

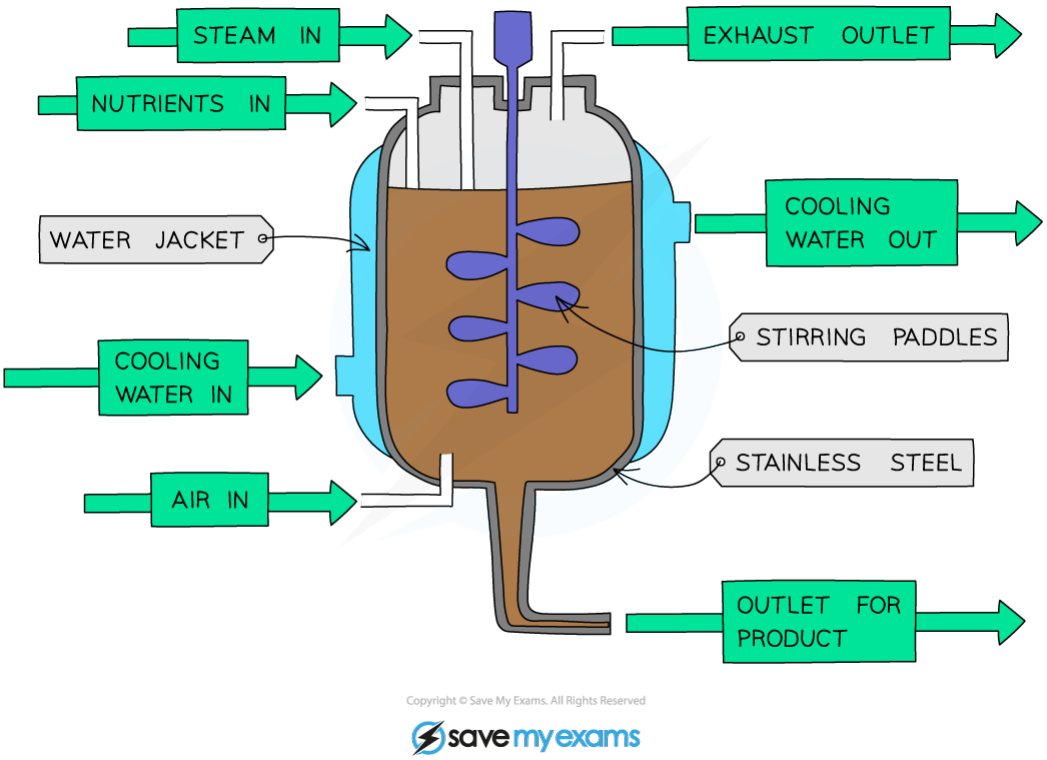
6.4 Cloning & Biotechnology

YOUR NOTES

Factor to be controlled	Explanation
pH	pH is monitored by probes and acids or alkalis added to provide optimum pH for enzyme activity. This means rates of reaction are maximised and consequently so is growth and productivity of the microorganisms
Temperature	Optimum temperatures are maintained using a water jacket. This facilitates maximum enzyme activity and therefore maximum yield
Oxygen availability	Sterile air is pumped in to provide optimum oxygen to maximise respiration and therefore maximise yield
Nutrient supply	Nutrients are added and circulated to ensure microorganisms can access the nutrients required for maximum yield
Agitation	Paddles are used to ensure even distribution of temperature, nutrients, pH and oxygen throughout the fermenter
Contamination	Contamination of the culture can lead to interspecific competition for nutrients, oxygen and space, this will reduce product yield. Fermenters must be steam cleaned between cultures to prevent contamination
Waste removal	Waste products produced by the microorganisms can have a negative effect on the growth and productivity of the microorganisms. Waste products are constantly removed to prevent these toxic effects

6.4 Cloning & Biotechnology

YOUR NOTES



A fermenter is carefully monitored and controlled to ensure optimum conditions for growth of microorganisms. This will maximise yield of the required product

YOUR NOTES ⬇

6.4 Cloning & Biotechnology

6.4.12 STANDARD GROWTH CURVE OF MICROORGANISMS

Standard Growth Curve of Microorganisms

- Bacterial colonies can **grow rapidly** when in culture with very large numbers of bacteria produced within hours
- Populations of microorganisms, such as bacteria, can be measured in three different ways:
  - **Direct counting** includes all cells (both living and dead) and involves taking samples to count these individual microorganisms
  - **Viable counting** involves culturing samples of microorganisms and counting the colonies that grow. This method only takes **living cells** from the sample into account
  - **Turbidity** is a measure of living and dead microorganisms in solution, taking an **absorbance** reading using a **colorimeter**

Turbidity

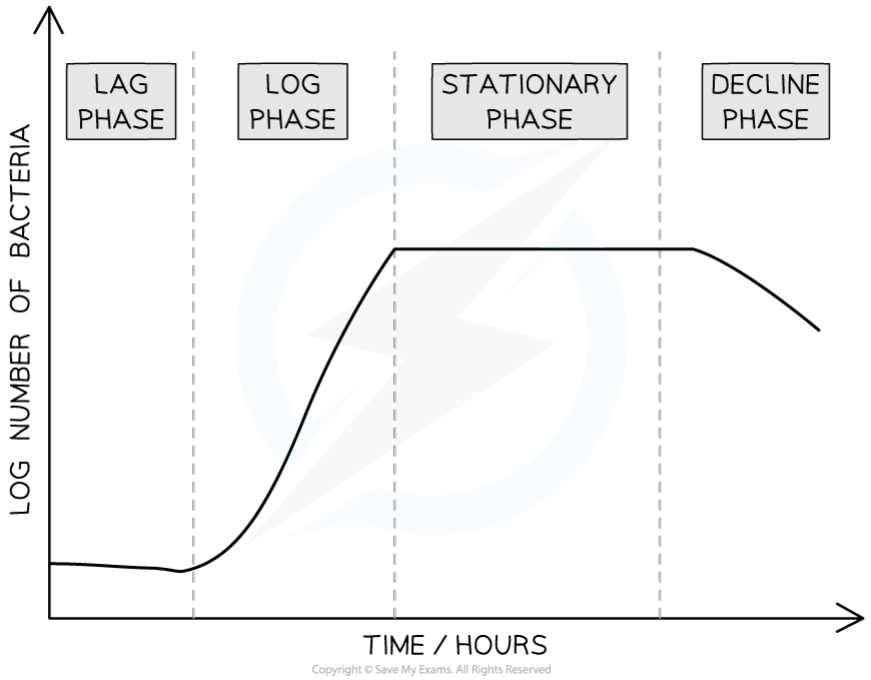
- Microorganisms, such as bacteria or yeast, can be grown in a **broth culture**
- Measuring the **turbidity** of this **suspension** can then be used as a way of estimating the number of cells (i.e. the **population size**) of the microorganisms in the broth culture
  - Turbidity is simply a measure of the **cloudiness** of a suspension (i.e. how much **light** can **pass through** it)
- As the microorganisms in the broth culture **reproduce** and their **population grows,** the suspension becomes progressively more **turbid** (cloudy)
- This changing turbidity can be monitored by measuring how much light can pass through the suspension at **fixed time intervals** after the **initial inoculation** of the **nutrient broth** with the microorganisms
  - A **turbidity meter**, a **light sensor** or a **colorimeter** (connected to a datalogger) can be used to take these measurements
- The results can then be used to plot a **population growth curve** to show how the population of microorganism grew over time

Standard population growth curves

- There are 4 phases in the population growth curve of a microorganism
  - **Lag phase** – the population size increases slowly as the microorganism population adjusts to its new environment and gradually starts to reproduce
  - **Log phase** – with high availability of nutrients and plenty of space, the population moves into exponential growth (the population doubles with each division)
  - **Stationary phase** occurs when the population reaches its maximum as it is limited by resources e.g. nutrients, toxic substances. The number of microorganisms dying equals the number being reproduced by binary fission
  - **Decline phase** occurs due to lack of nutrients and death due to toxic substance build up. Death rate exceeds reproduction rate

6.4 Cloning & Biotechnology

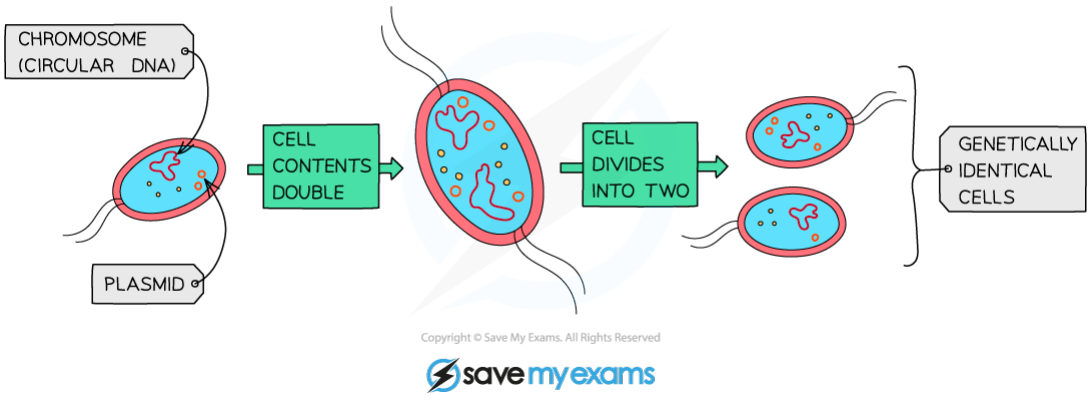
YOUR NOTES



There are 4 phases in the standard growth curve of a microorganism

Calculating population growth

- Bacteria divide using the process of **binary fission** where one cell will divide into two identical cells
- The process is as follows:
  1. The single, circular DNA molecule undergoes DNA replication
  2. Any plasmids present undergo DNA replication
  3. The parent cell divides into two cells, with the cytoplasm roughly halved between the two daughter cells
  4. The two daughter cells each contain a single copy of the circular DNA molecule and a variable number of plasmids



6.4 Cloning & Biotechnology

YOUR NOTES ⬇

The process of binary fission where a single cell divides into two identical daughter cells

- The following equation is used to calculate the rate of cell division by binary fission:

$$N = N_0 \times 2^n$$

  - **N** = the final number of bacteria
  - **N_0** = the initial number of bacteria
  - **n** = the number of divisions

Worked Example

A species of bacteria divides once every 25 minutes. Starting with a single cell, calculate how many cells there would be after 5 hours

Stage 1: Work out how many divisions there will have been in 5 hours

5 hours = 300 minutes

There is one division every 25 minutes

300 ÷ 25 = 12 divisions in 5 hours

Stage 2: Apply the equation $N = N_0 \times 2^n$

N_0 = 1

n = 12

N = 1×2^{12}

N = 4098 bacteria cells

Using logarithms in growth curves

- During the **exponential growth phase**, bacterial colonies can grow at rapid rates when in culture, with very large numbers of bacteria produced within hours
- Dealing with the experimental data relating to **large numbers** of bacteria can be difficult when using traditional linear scales
  - There is a wide range of very small and very large numbers
  - This makes it hard to work out a suitable scale for the axes of graphs
- **Logarithmic** scales can be very useful when investigating bacteria or other microorganisms

6.4 Cloning & Biotechnology

YOUR NOTES

Using logarithms to deal with orders of magnitude

- Logarithmic scales allow for a **wide range of values** to be displayed on a single graph
- For example, yeast cells were grown in culture over several hours. The number of cells increased very rapidly from the original number of cells present
- The results from the experiment are shown in the graph below, using a log scale
  - The number of yeast cells present at each time interval was converted to a logarithm before being plotted on the graph
  - The log scale is easily identifiable as there are **not equal intervals** between the numbers on the y-axis
  - The **wide range of cell numbers fit** easily onto the same scale

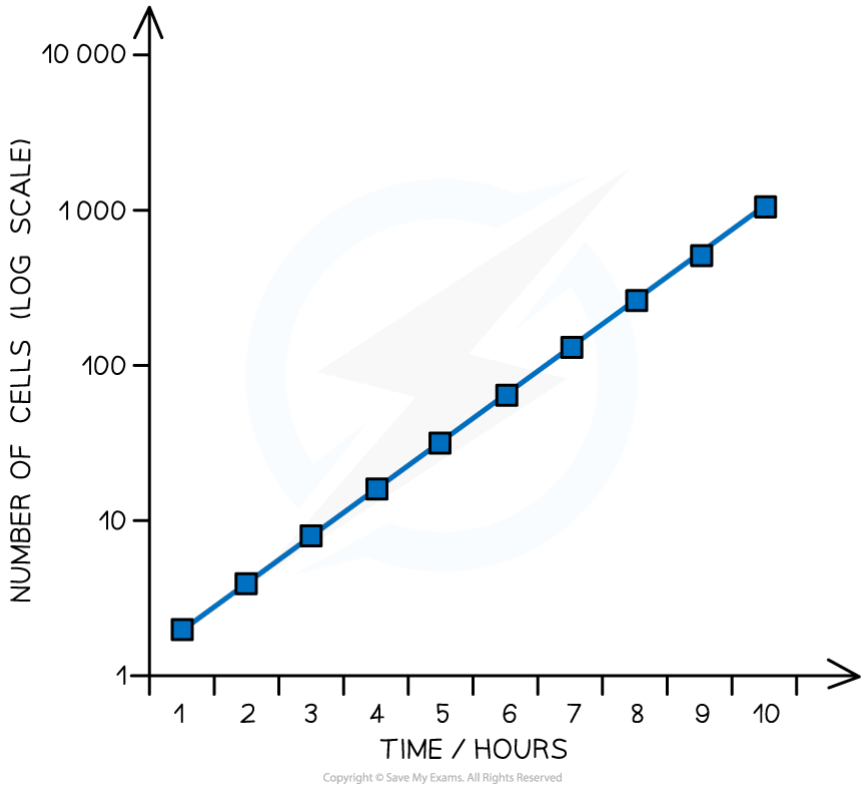


Image showing the number of yeast cells grown in culture over 10 hours, using a logarithmic scale

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YOUR NOTES ⬇

Exam Tip

You won't be expected to convert values into logarithms or create a log scale graph in the exam. Instead you might be asked to interpret results that use logarithmic scales or explain the benefit of using one! Remember that graphs with a logarithmic scale have **uneven intervals** between values on one or more axes.

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

6.4.13 FACTORS AFFECTING THE GROWTH OF MICROORGANISMS

Practical: Factors Affecting the Growth of Microorganisms

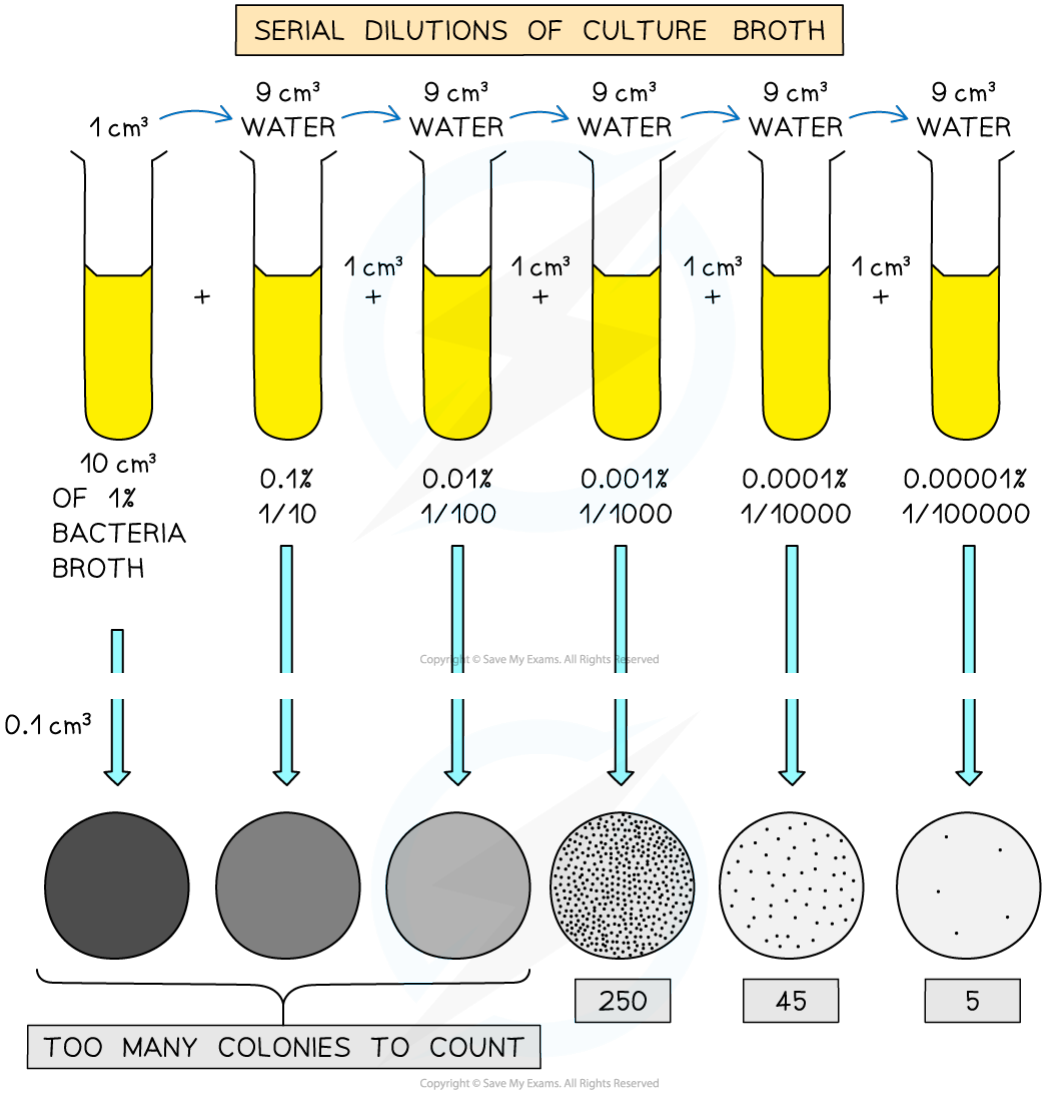
- To investigate the **effect of different factors** e.g. temperature or pH, on the growth of microorganisms
- The agar plates are prepared, using the same procedure as discussed in 6.4.10, to **avoid contamination**
- After the bacteria have been cultured in the desired conditions, it may become apparent that they are **too numerous to count** as there are so many colonies or the colonies **overlap**, forming a **lawn**
- A solution to this problem is to use a **serial dilution** method to dilute the bacteria in the broth before plating them onto the agar

Serial dilutions

- Serial dilutions are created by taking a series of dilutions of a stock solution (sample of the microorganism culture broth). The concentration decreases by the **same** quantity between each test tube
    - They can be created to any chosen dilution factor, but most commonly, either by 'doubling dilutions' (where the concentration is halved between each test tube) or a desired range (e.g. 0, 2, 4, 6, 8, 10 mmol dm^{-3})
- Serial dilutions are completed to create a less dense culture of cells.
    - This means more countable colonies when studying bacteria or yeast populations

6.4 Cloning & Biotechnology

YOUR NOTES



In order to study cultured microorganisms, it might be necessary to produce a serial dilution so that colonies are easier to see.

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

Investigating the effect of temperature on the growth of microorganisms

Apparatus

- Sterile agar plates
  - The agar can be made sterile by boiling
- Diluted bacterial broth with a concentration of 1 x 10^8 CFU mm^{-3}
  - Colony-forming unit (CFU): a live bacterial cell that is able to reproduce and form a colony on the agar plate
- Pipettes
- Spreaders
- Bunsen burner
- Gloves
- Goggles
- Incubator
- Fridge

Method

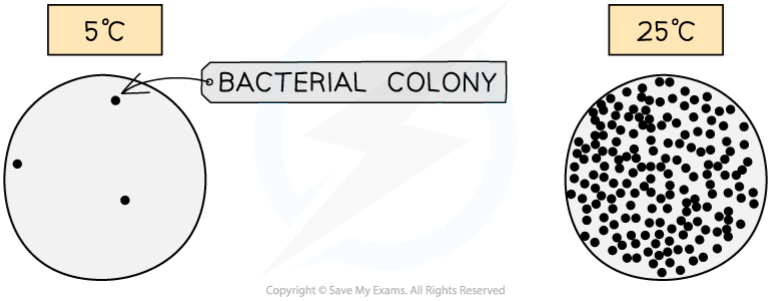
- Spread a sample of the diluted bacterial broth onto the surface of each of the sterile agar plate
- Tape the lid shut
- Keep three of the agar plates in the fridge overnight 5°C
- Keep three of the agar plates in the incubator overnight at 25°C
- Remove the agar plates the next morning and count the number of colonies, keeping the lid on as you count
- Calculate the average number of colonies that have formed and compare the results at each temperature

Results

- Overnight, the bacterial colonies will grow large enough that they can be easily counted
- The bacteria that were cultured at **25°C** are expected to have developed at a much **faster rate** with many more colonies visible
- The bacteria cultured at **5°C** are expected to have formed **fewer** colonies which are much **smaller** in size, or even no colonies at all
- This difference is due to the fact that 25°C provides a temperature close to the **optimum for enzyme activity** in the bacteria
- As a result, the rate of growth is much faster than those cultured at 5°C as enzymes' cellular reactions will be very slow

6.4 Cloning & Biotechnology

YOUR NOTES



These results show the difference in bacterial colony growth at 5°C compared to 25°C

Variations on this investigation

- We can investigate the effect of **pH** and **nutrient** availability using a very similar method to the one detailed above.
  - pH can be altered using different buffer solutions with different pH levels to the broth
  - Nutrient availability can be altered by using different agar plates with different nutrient contents
- It is also possible to use **turbidity** as a measure of growth instead of counting the colonies

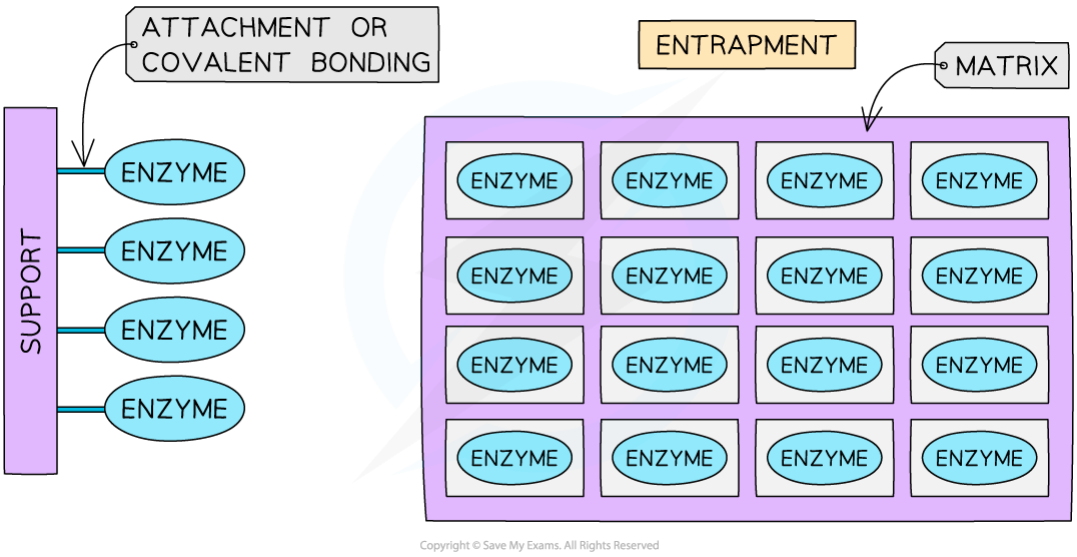
6.4 Cloning & Biotechnology

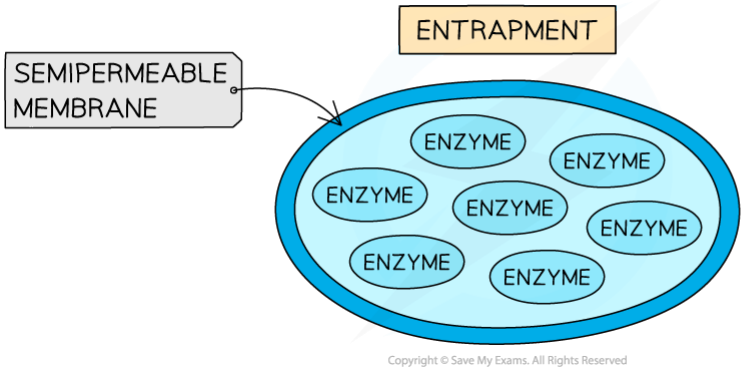
YOUR NOTES

6.4.14 IMMOBILISED ENZYMES IN BIOTECHNOLOGY

Immobilised Enzymes in Biotechnology

- An **immobilised enzyme** is an enzyme that is attached to an **insoluble material** to prevent mixing with the product
- Enzymes can be immobilised in several ways:
  - Attached to an **inert substance**
  - Enclosed in a **capsule**
  - Contained within a **partially permeable membrane**



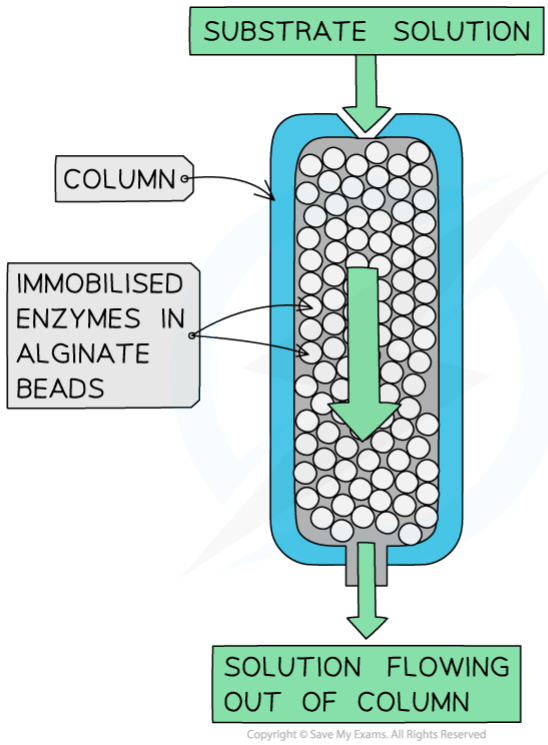


Different ways in which enzymes can be immobilised

6.4 Cloning & Biotechnology

YOUR NOTES

- Enzymes are often immobilised for use in **industrial processes** as it means the enzyme can be **reused** in future processes rather than being discarded after it has been used once
- Reusing the enzyme also **avoids the need to separate** the enzyme from the product in downstream processing
    - The immobilised enzymes are contained within a **column** through which the substrate is filtered in **solution**
    - As the substrate runs through the column, **enzyme-substrate complexes** are formed and products are produced
    - These products then flow out of the column, **leaving the enzymes behind** to catalyse the reaction again



The immobilised enzymes are contained within a column

Advantages of immobilised enzymes

- There is **no enzyme in the product** (the product is **uncontaminated**) and therefore there is no need to further process or filter the end product
- The immobilised enzyme can be **reused multiple times** which is both efficient and **cost-effective** (enzymes are **expensive**)
- Immobilised enzymes have a **greater tolerance of temperature and pH changes** (immobilisation often makes enzymes more **stable**)

6.4 Cloning & Biotechnology

YOUR NOTES ⬇

Disadvantages of immobilised enzymes

- Specialist **expensive equipment** is required
- Immobilised enzymes are **more costly to buy**, so are unlikely to be financially worthwhile for **smaller industries**
- The **rate of reaction is sometimes lower** when using immobilised enzymes as the enzymes cannot freely mix with the substrate

Immobilised enzymes in industry

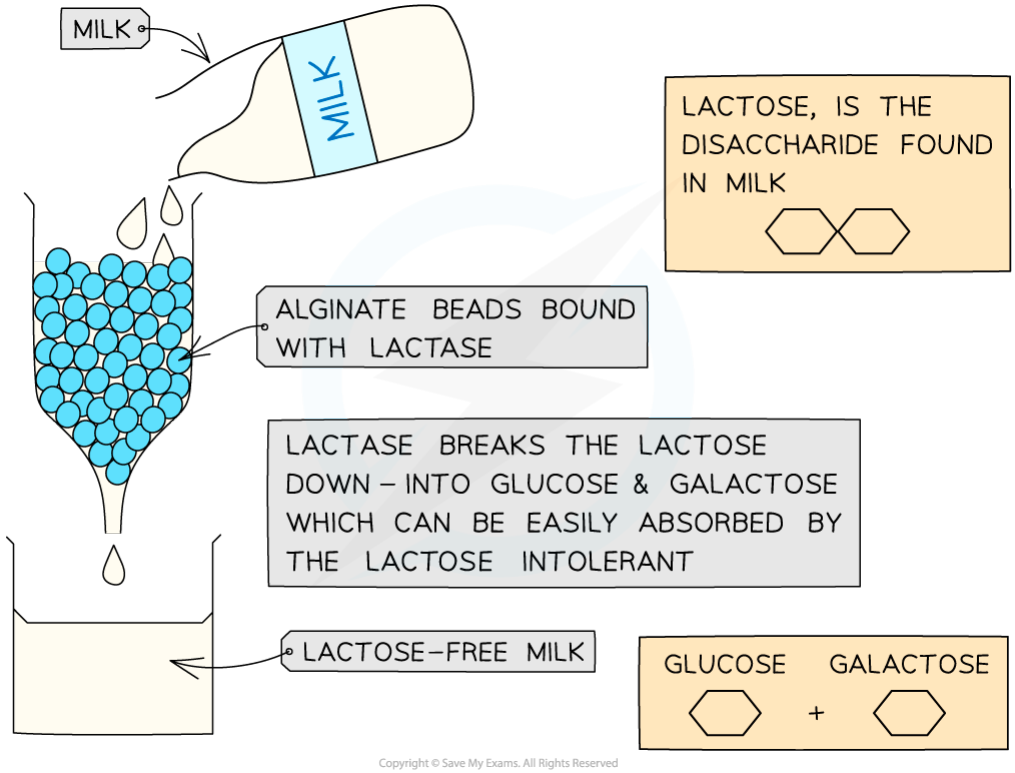
- There are many **industrial** and **medical** applications of immobilised enzymes, including production of the following:
  - **Lactose-free** dairy products such as milk
    - Enzyme: Lactase
    - Converts lactose to glucose and galactose
  - **Semi-synthetic penicillin** which overcomes issues of penicillin resistance
    - Enzyme: Penicillin acylase
    - Converts the original form of penicillin into one which is effective against penicillin-resistant organisms
  - **Glucose** products used to sweeten and thicken foods
    - Enzyme: Glucoamylase
    - Converts starch and other dextrins into glucose
  - **Fructose** for sweetening of foods where a lower quantity of sugar is necessary
    - Enzyme: Glucose isomerase
    - Converts glucose into the sweeter sugar, fructose
  - Purified samples of **L-amino acids** used in food production
    - Enzyme: Aminoacylase
    - Separates out L-amino acids from D-amino acids
  - **Acrylamide** required in disposable nappy production
    - Enzyme: Nitrilase
    - Converts acrylonitrile into acrylamide

A closer look at lactose-free milk production

- **Milk** is a valuable source of nutrients containing protein, fat and the carbohydrate lactose
- 5-10% of the UK population are **lactose intolerant**
- Lactose is a **disaccharide** that is broken down into **glucose and galactose**

6.4 Cloning & Biotechnology

YOUR NOTES



Using immobilised enzyme to modify milk

Exam Tip

You will not necessarily be asked about these specific examples of industrial uses of immobilised enzymes, but it is useful to know of some uses in order to be able to apply your knowledge accurately in the exam.

When discussing the advantages and disadvantages of immobilised enzymes, try to be specific about the cost implications as there are various considerations when it comes to the economical value of immobilising the enzymes.