

# Biotechnology and genetic modification

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## What you need to know

Biotechnology means using living things, usually microorganisms, or the enzymes they make, to make products people want. Genetic modification (GM) is one tool it uses. This topic turns up on every paper: nearly always the last one or two multiple-choice questions on Papers 1 and 2, and parts of a longer question on Papers 3 and 4. By the end of it you should be able to:

### Why bacteria are used

1. Give the two reasons bacteria are such good workers for industry and GM: they **reproduce rapidly** and they can **make complex molecules**.
2. **Extended only:** add two more: hardly anyone objects to growing and altering them (**few ethical concerns**), and they carry **plasmids**.

### Biotechnology

3. Explain how yeast, respiring **without oxygen**, turns sugar from plants into **ethanol** that is burned as a **biofuel**.
4. Explain what yeast does in dough to make **bread** rise.
5. Explain what **pectinase** does to crushed fruit and why juice makers add it.
6. Say which enzymes a **biological washing powder** contains and what each one cleans off, and plan and explain a test of how well such a powder removes stains.
7. **Extended only:** explain how the enzyme **lactase** gives milk that people who can't digest milk sugar can drink (**lactose-free milk**).
8. **Extended only:** explain how a big closed steel tank, a **fermenter**, grows huge numbers of microbes to make **insulin**, **penicillin** and **mycoprotein**.
9. **Extended only:** for each thing a fermenter keeps in check (**temperature**, **pH**, **oxygen**, **nutrient supply** and **waste products**), say why it matters to the microbes and how it is kept right.

### Genetic modification

10. Describe **genetic modification** as changing an organism's genetic material by **removing**, **changing** or **inserting individual genes**.

11. Give four uses of GM: bacteria that carry a human gene and **make a human protein**; crops given a gene so that **weedkiller doesn't harm them**, so that **insects that eat them die**, or so that they are **more nutritious**.
12. **Extended only**: list, in order, how a bacterium is given a human gene so that it makes the human protein: cutting out the gene with **restriction enzymes** (leaving **sticky ends**), cutting a **plasmid** with the same enzymes, joining them with **DNA ligase** to make a **recombinant plasmid**, putting it into bacteria, letting the bacteria **multiply**, and the bacteria **expressing** the gene to make the protein.
13. **Extended only**: weigh up the good and bad sides of growing GM crops such as **soya, maize and rice**.

How exactly a plasmid gets into a bacterium is not needed. Anaerobic respiration itself is in Respiration, and how enzymes work (active sites, denaturing) is in Enzymes.

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## Understand it

### Why bacteria are so useful

Bacteria are tiny single cells with no nucleus. Their DNA is a single large loop, and most also carry one or more **plasmids**: small extra rings of DNA. Four features make them the favourite workers of biotechnology.

- **They reproduce very fast.** A bacterium splits in two, and in good conditions it can do this every 20 minutes. One cell becomes  $2^6 = 64$  cells after 2 hours and over 16 million ( $2^{24} = 16\,777\,216$ ) after 8 hours. So a small starter culture quickly becomes a huge population that makes a lot of product.
- **They can make complex molecules**, such as proteins (insulin is a protein), from simple ones like sugar and ammonium ions.
- **Extended only: few ethical concerns.** Most people don't object to growing, changing or killing bacteria, as they would for animals.
- **Extended only: plasmids.** A plasmid is easy to take out, cut open, add a gene to, and put back into a bacterium. It then carries the new gene into the cell, and it is copied every time the cell divides.

One more fact explains why the whole idea works: **all living things use the same genetic code**. A human gene inside a bacterium is read in the same way as in a human cell, so the bacterium makes exactly the human protein.

Wrong reasons that appear as distractors: "they reproduce slowly", "they have a cell wall", "they have a cell membrane" or "a nucleus", "they are all harmless", "they cause decomposition", "they are not affected by antibiotics". A cell wall and a membrane are real, but they are not why bacteria are useful.

### Yeast: bread and biofuel

**Yeast** is a single-celled **fungus**. When it has sugar but little or no oxygen, it respire **anaerobically**:



**Extended only**: the balanced equation is  $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2$ .

People use **both products**, in different industries.

**Bread-making (the carbon dioxide matters).** Flour, water, sugar and yeast are mixed into dough and left somewhere **warm**. The yeast's enzymes break down starch and sugar, and the yeast respire anaerobically inside the dough. The **carbon dioxide** makes bubbles that are trapped in the dough, so it **rises** and the bread ends up light. When the bread is baked, the heat **kills the yeast**, the gas bubbles expand, and the little **ethanol** made **evaporates**.

**Biofuel (the ethanol matters).** Plant material rich in sugar or starch (sugar cane, maize, crop waste) is crushed and treated, often with enzymes, to release **glucose**. Yeast is added and kept **without oxygen**, so it respire anaerobically and makes **ethanol**. The ethanol is separated (distilled) from the mixture and burned as a fuel, on its own or mixed with petrol.

Why ethanol is a better fuel for the environment: the plants can be regrown, so it is **renewable** (it doesn't run out like oil); the carbon dioxide released when it burns was taken in by the plants for **photosynthesis**, so there is less **overall** increase in carbon dioxide than from fossil fuels; and it can use **waste** plant material. It is not true that burning ethanol gives off no carbon dioxide.

Yeast is also used for **brewing** (beer and wine), which again uses the ethanol.

## Enzymes in industry

Enzymes from microorganisms are made in large amounts and used on their own, without the living cells.

**Pectinase and fruit juice.** Plant cells are held together by **pectin**, a substance in their cell walls. Pectinase **breaks down pectin**, so when it is added to crushed fruit:

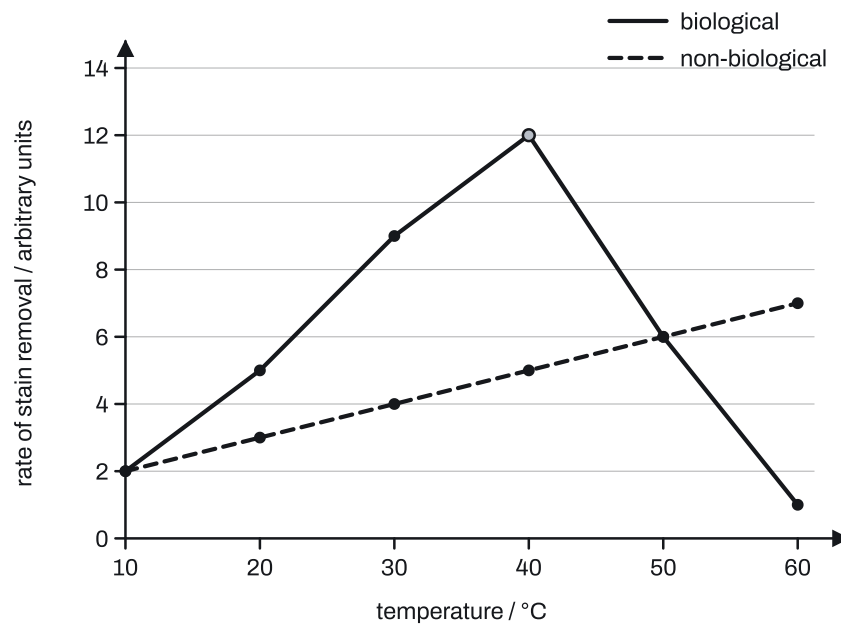
- the cells separate and their walls break down more easily, so **more juice is released** (a larger volume from the same mass of fruit);
- the juice is **clearer** (less cloudy), because the pectin that made it cloudy is broken down.

Pectinase works best at its optimum temperature and pH, like any enzyme. Juice extraction is often speeded up by also adding cellulase, which breaks down cellulose in cell walls.

**Biological washing powders.** These contain enzymes that digest the large, insoluble molecules in stains into small, **soluble** molecules that wash out in water:

| Enzyme          | Stain it removes                                | What it makes            |
|-----------------|-------------------------------------------------|--------------------------|
| <b>protease</b> | protein stains: blood, egg, grass, milk         | amino acids              |
| <b>lipase</b>   | fat and oil stains: butter, grease, cooking oil | fatty acids and glycerol |
| <b>amylase</b>  | starch stains: sauce, potato, pasta             | sugars (maltose)         |

Because the enzymes do the work, the powder cleans well at **low temperatures** (about 30–40 °C), which **saves energy** and doesn't damage delicate fabrics. Above about 50–60 °C the enzymes **denature** (their active sites change shape), so the powder works worse than at 40 °C. Washing powders are **alkaline**, so the enzymes chosen have a high optimum pH. For a mixed stain you need more than one enzyme.



The graph shows why: the biological powder is best at **40 °C**, its enzymes' **optimum**, where it removes stains  $12 \div 5 = 2.4$  times as fast as the non-biological powder. Above that it falls as the enzymes denature. The non-biological powder has no enzymes, so it just works a little better the hotter the water.

**Investigating a washing powder** (Core): cut equal squares from one cloth, stain each with the same volume of the same stain (egg, for example) and let them dry. Put each in the same volume of water with the same mass of powder, at each temperature, and time how long the stain takes to disappear, or measure how much is left after a fixed time. Use a **control** with **boiled powder** (its enzymes are denatured) or a non-biological powder, so you can see the enzymes are responsible. Repeat each temperature and find a mean. The rate is  $\frac{1}{\text{time}}$ : the shorter the time, the faster.

**Extended only: lactase and lactose-free milk.** Milk contains the sugar **lactose**. Some people (and pets) make little **lactase**, so lactose passes undigested into the large intestine, where bacteria ferment it and cause gas, pain and diarrhoea: they are **lactose intolerant**. **Lactase** breaks lactose down:



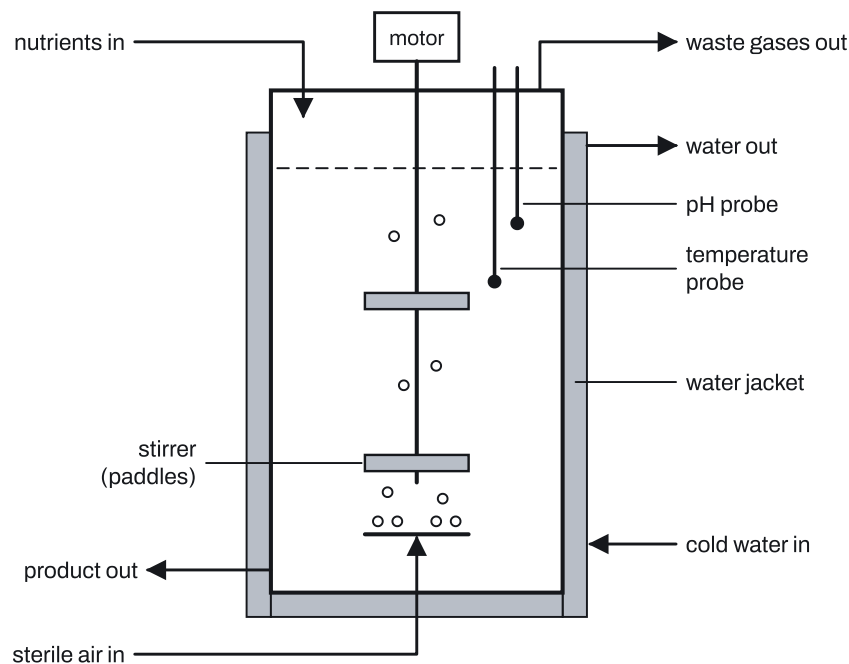
Milk is passed over lactase, or lactase is added to it, and the product is **lactose-free milk**, which tastes slightly sweeter. Often the enzyme is **immobilised**: trapped in small beads of **alginate** in a column, so milk flows through, the lactase stays behind to be used again, and none ends up in the milk. Lactose-free milk is made with an **enzyme**, not by growing a microorganism.

## Fermenters

**Extended only: A fermenter** is a large, closed steel tank for growing one microorganism in huge numbers, in exactly the right conditions, to make one product. Products include:

- **insulin** (for people with type 1 diabetes), made by **genetically modified bacteria**;
- **penicillin**, an antibiotic, made by the fungus **Penicillium**;

- **mycoprotein**, a high-protein meat substitute, made from the fungus **Fusarium**;
- ethanol, enzymes and other proteins.



Every condition is **monitored** by probes and a computer, and **controlled** to keep it at the best value, so the microorganisms grow fast and make as much product as possible.

| Condition              | Why it matters                                                                                                                                                   | How it is controlled                                                                                                 |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| <b>temperature</b>     | the microorganisms' respiration <b>releases heat</b> ; too hot and their <b>enzymes denature</b> , too cold and they grow slowly                                 | a temperature probe <b>monitors</b> it; cold water in the <b>water jacket</b> carries heat away                      |
| <b>pH</b>              | enzymes work best at their <b>optimum pH</b> ; carbon dioxide from respiration makes the culture more <b>acidic</b>                                              | a pH probe monitors it; acid or alkali is added, or a <b>buffer</b> keeps it steady                                  |
| <b>oxygen</b>          | needed for <b>aerobic respiration</b> , which releases the energy for growth (penicillin and mycoprotein fungi need a lot)                                       | <b>sterile air</b> is bubbled in at the bottom                                                                       |
| <b>nutrient supply</b> | <b>glucose</b> (or another sugar) for respiration; <b>amino acids</b> or <b>ammonium / nitrate ions</b> as the nitrogen source for making proteins; mineral ions | nutrients are <b>sterilised</b> , then added through a pipe, more being added as they are used                       |
| <b>waste products</b>  | carbon dioxide and other wastes build up and are <b>toxic</b> , and lower the pH                                                                                 | waste gases leave through an <b>outlet (vent)</b> , which also stops the pressure rising; the product is drained off |

Two more features are about the whole process:

- **Stirring** with the paddles spreads **nutrients, oxygen, heat and microorganisms evenly** through the tank, so every cell gets what it needs and there are no hot spots.
- **Everything is sterile.** The tank is cleaned with steam before use, and the nutrients and air are sterilised. Any other microorganism that got in would **compete** for the nutrients, could make **toxins** or spoil the product, and would lower the **yield**.

**Growth in a fermenter** follows the usual curve for a population (see Nutrient cycles and populations): a **lag phase** (cells adjusting, little growth), an **exponential (log) phase** (fast growth), a **stationary phase** (growth stops because nutrients are running out and toxic wastes are building up) and, if nothing is added, a **death phase**. Penicillin is made **mainly after the fast growth**, when the fungus is in the stationary phase and sugar is running low, so graphs show penicillin rising late, while the sugar falls.

After the run, the liquid is **filtered** to remove the microorganisms, and the product is **extracted and purified** (then, for penicillin, made into tablets or injections).

## Genetic modification

**Genetic modification** (also called genetic engineering) means changing the genetic material of an organism by **removing, changing or inserting individual genes**. It is different from:

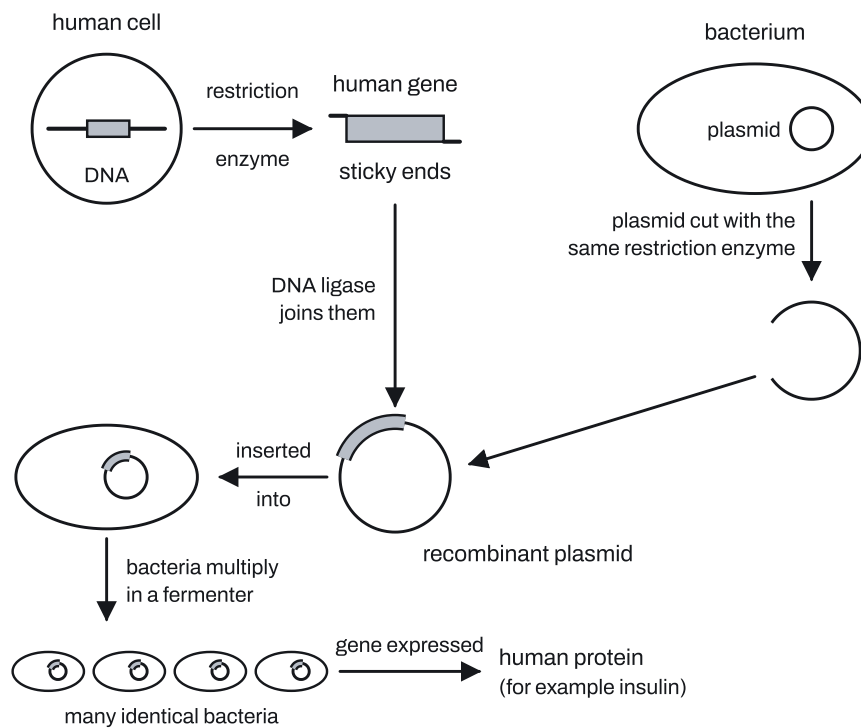
- **selective breeding**, which chooses which parents breed so that useful alleles are passed on over many generations; no gene is moved;
- a **mutation**, which is a random change;
- adding vitamins to food, or using yeast to make bread.

### Examples to learn:

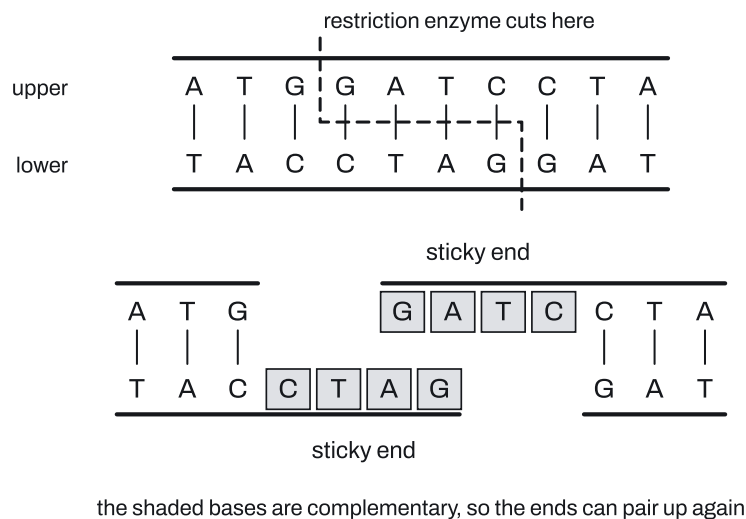
- **Human proteins made by bacteria:** the human **insulin** gene is put into bacteria, which then make human insulin for people with diabetes. Human growth hormone and blood-clotting proteins are made the same way.
- **Crops resistant to herbicides:** the crop gets a gene that stops a weedkiller harming it. The farmer can spray the field, killing only the **weeds**, so the crop has less competition and gives a **higher yield**.
- **Crops resistant to insect pests:** a gene from a bacterium makes the crop produce a **toxin** that kills insects that eat it. Less damage, higher yield, and **less insecticide** needs to be sprayed.
- **Crops with better nutritional qualities:** for example **golden rice** makes **beta-carotene**, which the body turns into **vitamin A**, helping people whose diet lacks it (vitamin A deficiency causes blindness).

## How a bacterium is made to produce a human protein

**Extended only:** Learn these six steps in order: examiners ask for the order, for the name of each enzyme, and for full descriptions.



1. **Isolate the gene.** The DNA making up the human gene is **cut out** of a human chromosome with a **restriction enzyme**. The enzyme cuts at a particular **base sequence**, and it cuts the two strands at slightly different places, so each end is left with a short single-stranded run of bases: a **sticky end**.
2. **Cut the plasmid.** A plasmid from a bacterium is cut open with the **same restriction enzyme**. It cuts the same base sequence, so the plasmid's sticky ends have bases that are **complementary** to the gene's sticky ends.
3. **Join them.** The gene is mixed with the opened plasmids. The complementary sticky ends pair up (A with T, C with G), and the enzyme **DNA ligase** joins the DNA backbones permanently. The plasmid with the human gene in it is a **recombinant plasmid**.
4. **Put it into bacteria.** The recombinant plasmids are taken up by bacteria. (You don't need the details.)
5. **Multiply.** The bacteria are grown in a **fermenter**, where they reproduce rapidly, and every new cell has copies of the recombinant plasmid.
6. **Express the gene.** The bacteria read the human gene and **make the human protein**, which is then extracted and purified.



The second diagram shows why the **same** enzyme must cut both: the enzyme cuts only where it finds its own base sequence (here G G A T C C). Its **active site** fits only that sequence, just as any enzyme's active site fits one substrate. Every cut leaves the same overhang, so any two pieces cut by it have **complementary** sticky ends that can base-pair and be joined. A different enzyme would leave ends that don't match.

Keep the two enzymes apart: **restriction enzymes cut** DNA; **DNA ligase joins** it. (Ligase, not lipase: lipase digests fat.) What goes into the bacterium is the **recombinant plasmid**, not the human chromosome, and not insulin.

## GM crops: for and against

**Extended only:** Soya, maize and rice are the crops most often modified. You need both sides.

| Advantages                                                                                                                   | Disadvantages                                                                                                                                 |
|------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| <b>higher yield</b> from the same land, as fewer plants are lost to pests and weeds                                          | <b>GM seed is expensive</b> , and farmers may have to <b>buy new seed every year</b> from a few large companies                               |
| pest-resistant crops need <b>less insecticide</b> : cheaper, and fewer harmless insects (such as pollinators) are killed     | pollen could carry the gene to <b>wild relatives</b> , making <b>herbicide-resistant weeds</b> that are hard to kill                          |
| herbicide-resistant crops let <b>weeds be killed</b> without harming the crop, so less competition for light, water and ions | insect pests could <b>evolve resistance</b> to the toxin, so it stops working                                                                 |
| <b>better nutrition</b> , such as golden rice for vitamin A, helping to prevent deficiency diseases                          | spraying kills all the weeds and the toxin may kill harmless insects, so <b>biodiversity falls</b> ; GM crops are often grown as monocultures |
| crops can be made to resist <b>disease, drought, salty soil</b> or cold, or to <b>last longer</b> before spoiling            | possible <b>unknown effects on health</b> (for example allergies) and on food chains; some people have <b>ethical</b> objections              |

**Before a GM crop is sold**, scientists breed it for many generations, to check that: the new gene is **inherited** by the offspring and still **expressed** (every plant is resistant); the **yield and quality** are good; it grows well in the **different places** it will be farmed; the gene has **no unexpected or harmful effects**; and to build up a large stock of **seed** to sell.

**Stopping GM pollen reaching wild plants:** grow the crop far from wild relatives or in **glasshouses**; **cover the flowers**; **remove the anthers** (or use sterile plants); or plant a border of a different species around the crop.

## Key facts and formulas

"Learn it" means you must remember it: the exam gives no formulas for this topic.

| Formula                                                                                                                                                           | Status   |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Bacteria: rapid reproduction, make complex molecules; Extended: few ethical concerns, plasmids                                                                    | Learn it |
| Yeast anaerobic respiration: glucose → ethanol + carbon dioxide                                                                                                   | Learn it |
| Extended: $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$                                                                                                            | Learn it |
| Bread: carbon dioxide makes the dough rise; biofuel: ethanol is the fuel                                                                                          | Learn it |
| Pectinase breaks down pectin: more juice, clearer juice                                                                                                           | Learn it |
| Protease → protein stains; lipase → fat and oil stains; amylase → starch stains                                                                                   | Learn it |
| Extended: lactase breaks down lactose → glucose + galactose, giving lactose-free milk                                                                             | Learn it |
| Extended: fermenter conditions: temperature, pH, oxygen, nutrients, waste products; plus stirring and sterile conditions                                          | Learn it |
| GM: changing genetic material by removing, changing or inserting individual genes                                                                                 | Learn it |
| Extended: restriction enzyme cuts (sticky ends) → same enzyme cuts plasmid → DNA ligase joins → recombinant plasmid → into bacteria → multiply → express the gene | Learn it |
| Percentage change = $\frac{\text{new} - \text{original}}{\text{original}} \times 100$                                                                             | Learn it |
| Rate = $\frac{1}{\text{time}}$                                                                                                                                    | Learn it |

## How to do it

We tagged 133 questions for this topic (2021–2025, Papers 1–4; 123 different questions, as 10 are repeats of a question on another paper). 20 of them mix more than one type, so the counts add up to 165.

| Question type                                                                                  | Questions |
|------------------------------------------------------------------------------------------------|-----------|
| State why bacteria (and plasmids) are useful in biotechnology and GM                           | 43        |
| Define GM, give its uses in crops and for human proteins, and weigh up GM crops                | 32        |
| Uses of yeast's anaerobic respiration: bread, biofuel (ethanol), equation                      | 23        |
| Describe the steps of GM: restriction enzymes, sticky ends, ligase, recombinant plasmid, order | 22        |
| Enzymes in industry: pectinase (fruit juice), washing powders, lactase                         | 21        |
| Read, describe or calculate from biotechnology data (graphs, tables)                           | 13        |
| Fermenters and penicillin: conditions controlled and why, growth phases                        | 11        |

### 1. State why bacteria (and plasmids) are useful in biotechnology and GM (43 questions)

1. **Core, "state two reasons": rapid reproduction rate** and the **ability to make complex molecules**. Use those words: "they reproduce rapidly", not "they grow fast".
2. **Extended** adds: **few ethical concerns** over growing and changing them; they have **plasmids**; they share the **same genetic code** as other organisms, so a human gene makes the human protein.

#### Variations you will meet:

- **Multiple choice with numbered statements:** pick "make complex molecules" and "reproduce rapidly". Reject slow reproduction, cell walls, cell membranes, a nucleus, "unaffected by antibiotics", "have no plasmids", "all harmless" and "cause decomposition".
- **A bacterium diagram: "which structure is useful in GM?"** or "which part is cut to form sticky ends?": the **plasmid**, the small ring, not the large loop of DNA.
- **"Apart from structural features, state two reasons":** plasmids are a structure, so give rapid reproduction, complex molecules and few ethical concerns instead.
- **A table of bacteria:** choose the one with the **highest reproduction rate** (most divisions per hour) **and a high** ability to make complex molecules.
- **"Which organisms have plasmids?": bacteria.**
- **"With which kingdoms do bacteria share the same genetic code?": all of them** (animals, plants, fungi and protocists).
- Don't list **products** (insulin, yoghurt) when the question asks for **reasons**.

## 2. Define GM, give its uses in crops and for human proteins, and weigh up GM crops (32 questions)

1. **Define:** changing the **genetic material** of an organism by **removing, changing or inserting individual genes**. It applies to any organism, not to plants or animals only.
2. **Examples** (1 mark each): bacteria making **human insulin** or other human proteins; crops **resistant to herbicides**; crops **resistant to insect pests**; crops with better **nutritional qualities** (golden rice, beta-carotene).
3. **"Describe how GM can improve crop plants"** (3 marks): herbicide resistance, insect or pest resistance, higher yield, better nutrition; also disease or drought resistance and longer shelf life.
4. **Extended, "discuss the advantages and disadvantages"**: give **both sides**, each point with its reason, using the table in "Understand it". Say **seeds** are expensive (not "the plants"), and add that farmers must buy them every year.

### Variations you will meet:

- **Multiple choice "reasons GM is used in crops"**: less herbicide, less insecticide and less pest damage; **not** "reduces nutritional quality".
- **Name the purpose** of a new GM crop from its description: a pineapple with more of a healthy substance is **improving nutritional qualities**; a crop that survives weedkiller is **herbicide resistance**.
- **Is it GM?** Inserting a gene is GM. **Choosing** animals to breed (selective breeding), adding vitamins to food and using yeast are not.
- **A disadvantage of a herbicide-resistant crop**: the gene could pass to **weeds** by pollination, so the weeds also resist the herbicide.
- **Why breed GM plants for many generations first?** Check the gene is **inherited and expressed**, the **yield and quality** are good, it grows in **different environments**, there are **no unwanted effects**, and **build up seed**. Don't write about selective breeding or mutation.
- **Stop GM crops pollinating wild plants**: **glasshouses**, **isolate** the crop, **cover the flowers**, **remove anthers**.
- **Disadvantages of one GM crop, such as golden rice**: seed cost each year; **cross-breeding with wild rice**; fewer varieties grown, so less biodiversity; unknown long-term health effects. Keep to GM; don't drift into the problems of monocultures in general.

## 3. Uses of yeast's anaerobic respiration: bread, biofuel (ethanol), equation (23 questions)

1. **Word equation** (2 marks): **glucose** → **ethanol (alcohol) + carbon dioxide**.
2. **Extended, balanced equation**:  $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ .
3. **Match the product to the use**: **carbon dioxide makes bread dough rise**; **ethanol is the biofuel** (and the alcohol in brewing).

### Variations you will meet:

- **"Describe the role of anaerobic respiration in bread-making":** yeast respire anaerobically in the dough, making **carbon dioxide gas**, which is trapped as bubbles and **makes the dough rise**. The alcohol is **not** why yeast is used; it evaporates when the bread is baked.
- **"Which organism is used for both bread and biofuel?":** **yeast**. "Name another use of yeast": **biofuel**, **brewing** or bread-making (not just "baking" or "alcohol").
- **A biofuel flow chart:** plant biomass is broken down to release **glucose**; **yeast** respire it **anaerobically**; **ethanol** is extracted.
- **Why keep oxygen out of the biofuel tank?** With oxygen, yeast respire aerobically and makes carbon dioxide and water, **not ethanol**.
- **Extended, environmental advantages of ethanol fuel:** **renewable**, so it does not run out; the carbon dioxide released was **absorbed by the plants** in photosynthesis; it can use **waste** plant material; less need to drill for oil. Don't say it releases **no** carbon dioxide, and write "renewable", not "reusable".
- **Matching rows:** biofuel and bread use **yeast**; fruit juice uses **pectinase**.

#### 4. Describe the steps of GM: restriction enzymes, sticky ends, ligase, recombinant plasmid, order (22 questions)

1. **Extended, the 6-mark description:** write one step per sentence, in order, with the key words:
  - the human **gene** (a section of DNA) is **cut out** of the chromosome using a **restriction enzyme**;
  - the cut leaves **sticky ends**;
  - a bacterial **plasmid** is cut with the **same restriction enzyme**;
  - so the sticky ends are **complementary** (their bases pair);
  - the gene is **inserted into the plasmid**, joined by **DNA ligase**;
  - forming a **recombinant plasmid**;
  - the recombinant plasmid is **inserted into a bacterium**;
  - the bacteria **multiply** (in a fermenter) and **express** the gene, **making the human protein**.
2. **Put steps in order:** cut out the gene → cut the plasmid (these two can swap) → join with ligase → insert into the bacterium → the bacteria multiply → they make the protein → the protein is extracted.

#### Variations you will meet:

- **"Which stage uses DNA ligase?":** the stage where the gene is **inserted into the plasmid**. Restriction enzymes are used when the gene is removed and the plasmid is **cut open**.
- **Name enzymes A and B** on a flow diagram: A cuts both DNAs, so it is a **restriction enzyme**; B joins them, so it is **DNA ligase**. The product C is a **recombinant plasmid** (not "a GM organism").
- **"Why must the same enzyme cut both?"** It cuts the same base sequence, so both have **sticky ends with complementary bases**, which can pair and be joined.
- **"Explain how a restriction enzyme is specific to one base sequence":** its **active site** has a shape **complementary** to that sequence only, so it binds and cuts only there.
- **Complete sentences about a diagram** (such as golden rice made using bacteria): plasmid, restriction enzyme, sticky ends, gene, ligase, recombinant plasmid; tissue culture is a form of **asexual** reproduction.

- **"What is inserted into the bacteria?": recombinant plasmids** (not human plasmids, not insulin). **"What is the first step?":** cutting out the human gene with a restriction enzyme.
- **Statements about enzymes:** "restriction enzymes cut DNA" is correct; "ligase cuts DNA" and "ligase makes proteins" are wrong.

## 5. Enzymes in industry: pectinase (fruit juice), washing powders, lactase (21 questions)

1. **Name the enzyme for the job:** fruit juice → **pectinase**; oil or fat stain → **lipase**; protein stain (egg, blood) → **protease**; starch stain → **amylase**; lactose-free milk → **lactase** (Extended).
2. **Say what it does:** pectinase breaks down **pectin** in cell walls, so **more juice** is extracted and it is **clearer**; washing-powder enzymes digest **insoluble** stains into **soluble** products that wash away.
3. **Explain a temperature effect:** rate rises to the **optimum**; above it the enzymes **denature** (the active site changes shape), so stains are removed slowly or not at all. At low temperatures the molecules have less kinetic energy, so there are fewer successful collisions.

### Variations you will meet:

- **Multiple choice "why is pectinase useful?": more juice can be extracted.** Not cloudier juice, and pectinase doesn't digest proteins or starch.
- **"State one enzyme used in fruit juice production": pectinase** (amylase and cellulase can also be credited).
- **A graph of a washing powder against temperature:** it can show that the powder **contains enzymes** (it has an optimum, then falls). Don't claim it works best at all high temperatures, or that the rate is directly proportional to temperature.
- **Choosing an enzyme for a pH:** pick the one that is **active at that pH**, or whose optimum is **closest** to it.
- **Tick the correct conclusions** from a graph: tick only statements you can read from the data, and check each number against the axes.
- **Investigations** (juice volume or stain removal): name the **independent** variable (temperature, pH, enzyme concentration), the **dependent** variable (volume of juice, time to remove the stain) and the **controlled** variables (mass of fruit, volume of enzyme, time, temperature); use **boiled enzyme** or water as the control.
- **Extended, lactase:** breaks down **lactose** into **glucose and galactose**; for **lactose-intolerant** people; can be **immobilised** in alginate beads so the enzyme is reused and none is left in the milk. Lactose-free milk is made using an **enzyme**; insulin, penicillin and mycoprotein are made by growing microorganisms.

## 6. Read, describe or calculate from biotechnology data (graphs, tables) (13 questions)

1. **Read the values** from the graph or table, with the units and scale ("million tonnes"), and write them down.
2. **Calculate:** percentage change =  $\frac{\text{new} - \text{original}}{\text{original}} \times 100$ .
3. **Round as asked.** "Two significant figures" means keep the first two digits that count: 306.25 → 310, 0.04562 → 0.046.

For example, a crop harvest that rises from 45 to 131 million tonnes:  $\frac{131 - 45}{45} \times 100 = 191.11\%$ , which is 190% to two significant figures.

#### Variations you will meet:

- **"Describe the results"** (2–3 marks): the overall **trend**, where it **changes** (a peak, a levelling off), a **comparison** of two lines, and **data quotes with units**. With two y-axes, check which axis each line uses.
- **A proportion**: if 200 g of apples give 80 cm<sup>3</sup> of juice, then 350 g give  $\frac{350}{200} \times 80 = 140$  cm<sup>3</sup> in the same conditions.
- **Which bacterium gives the most product?** Multiply: a variety making 2.0 g of cells with 30% protein gives 0.6 g; one making 1.5 g with 50% protein gives 0.75 g, so the second is better.
- **Reading a value from your own graph**: draw lines on the graph to show where you read it.
- **Predicting at another temperature**: below the optimum, the enzymes of yeast or bacteria work more slowly, so the product (ethanol, juice) is made **more slowly** and less is made in the same time.
- **Finding the time** at which something reaches a value (for example half its maximum): read across from the y-axis to the line, then down.

## 7. Fermenters and penicillin: conditions controlled and why, growth phases (11 questions)

1. **Extended, "state the conditions and how they are controlled"** (up to 5 marks): say that each is **monitored**, then how it is kept right:
  - **temperature**: probe, and the **water jacket** removes heat from respiration;
  - **pH**: probe, and a **buffer** (or acid/alkali) because carbon dioxide lowers the pH;
  - both kept at the **optimum for the enzymes**;
  - **oxygen**: sterile air bubbled in, for aerobic respiration;
  - **nutrients**: glucose and a nitrogen source (amino acids or ammonium ions) added;
  - **waste**: carbon dioxide leaves through a **vent**, which also controls **pressure**;
  - **stirrer**: spreads nutrients, oxygen, heat and microorganisms **evenly**;
  - **sealed and sterile**, so no other microorganisms get in.

#### Variations you will meet:

- **"Which type of organism makes penicillin?"**: a **fungus** (*Penicillium*). "Name gas X that is bubbled in": **oxygen** (air).
- **"Why is air supplied?"**: for **aerobic respiration**, releasing energy for growth and reproduction.
- **"Why must the temperature be controlled?"**: respiration **releases heat**; too high and **enzymes denature**; too low and enzyme activity and growth are slow; keep it at the **optimum**.
- **"Why are the contents stirred?"**: to mix **nutrients, oxygen and microorganisms** and spread **heat** evenly.

- **"Why sterilise the nutrients?"**: to **kill microorganisms** that would **compete** with the fungus, spoil or contaminate the product, or lower the yield. "To keep it clean" is too vague.
- **"Why is there a gas outlet?"**: to let out **waste carbon dioxide** (it is toxic and lowers the pH) and stop the **pressure** rising.
- **"Name two nutrients"**: **glucose** (sugar) and **amino acids** (or ammonium or nitrate **ions**). Oxygen is not a nutrient.
- **"Name another product made in a fermenter"**: **insulin**, **mycoprotein**, ethanol.
- **Growth phases** on a curve: **lag**, **exponential (log)**, **stationary**, **death**. Growth stops because **nutrients are used up** and **toxic wastes build up**.
- **Penicillin graphs**: penicillin is made mostly **after** the fast growth, in the **stationary phase**, when sugar has run low.
- **Outline the steps after growth**: the fungus grows exponentially and makes penicillin; the liquid is **filtered** to remove the fungus; penicillin is **extracted**, purified and packaged.

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## Worked examples

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The bold codes show where marks are earned, as in Cambridge mark schemes. The number is how many marks.

- **M1** method mark: for a correct method, even if the numbers slip.
- **A1** accuracy mark: for a correct answer; it needs the M mark before it.
- **B1** mark for a correct result on its own, with no method needed.

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### Example 1

A baker mixes flour, water, sugar and yeast into dough and leaves it in a warm place for an hour.

- (a) State the type of organism that yeast is. **[1]**
- (b) Write the word equation for anaerobic respiration in yeast. **[2]**
- (c) Describe the role of anaerobic respiration in bread-making. **[2]**
- (d) State two reasons why microorganisms such as bacteria are useful in biotechnology. **[2]**
- (a) A fungus **B1**.
- (b) glucose → **B1** ethanol + carbon dioxide **B1**.
- (c) Yeast respire anaerobically in the dough, producing carbon dioxide **B1**; the gas is trapped as bubbles, so the dough rises **B1**.
- (d) They reproduce rapidly **B1**; they can make complex molecules **B1**.

## Example 2

Use the washing-powder graph in "Understand it". A student used the biological powder to remove egg stains.

- (a) State the optimum temperature for the biological washing powder. [1]
- (b) Explain why the rate of stain removal is lower at 60 °C than at 40 °C. [3]
- (c) Name the enzyme in the powder that removes the egg stain, and one other enzyme a biological powder contains, with the stain it removes. [2]
- (d) In a separate test, 250 g of crushed apple gave 24 cm<sup>3</sup> of juice without pectinase and 41 cm<sup>3</sup> with pectinase. Calculate the percentage increase in the volume of juice. Give your answer to two significant figures. [2]

(a) 40 °C **B1**.

(b) The powder contains enzymes **B1**; above the optimum the enzymes are denatured, their active sites change shape **B1**, so the stain (substrate) no longer fits and fewer enzyme–substrate complexes form, and the rate falls to 1 at 60 °C compared with 12 at 40 °C **B1**.

(c) Protease (egg is protein) **B1**; lipase removes fat or oil stains, or amylase removes starch stains **B1**.

(d)  $\frac{41 - 24}{24} \times 100 = 70.83 \dots$  **M1** = 71% **A1**

## Example 3

Human growth hormone is a protein. It can be made by bacteria that have been genetically modified.

- (a) Describe genetic modification. [2]
- (b) Outline how bacteria can be genetically modified to make human growth hormone. [6]
- (c) Explain why the same restriction enzyme must be used on the human DNA and on the plasmid. [2]
- (a) Changing the genetic material of an organism **B1**, by removing, changing or inserting individual genes **B1**.
- (b) Any six: the gene for growth hormone is cut out of human DNA **B1** using a restriction enzyme **B1**, leaving sticky ends **B1**; a plasmid is cut open with the same restriction enzyme **B1**; the gene is inserted into the plasmid and joined using DNA ligase **B1**, forming a recombinant plasmid **B1**; the recombinant plasmid is put into a bacterium; the bacteria multiply in a fermenter; they express the gene and make human growth hormone.
- (c) The enzyme cuts at the same base sequence in both, so the sticky ends are the same **B1**; the bases of the sticky ends are complementary, so they pair up and the gene fits into the plasmid **B1**.

## Example 4

A company grows a genetically modified fungus in a fermenter to make an enzyme.

- (a) Explain why air is bubbled into the fermenter and why the contents are stirred. [3]
- (b) Describe how the temperature and pH in the fermenter are kept at their best values. [4]
- (c) Over 15 years, the area of GM maize grown in one country rose from 36 to 95 thousand hectares. Calculate the percentage increase. Give your answer to two significant figures. [2]
- (d) Discuss the advantages and disadvantages of growing GM maize that is resistant to insect pests. [4]
- (a) Air provides oxygen for aerobic respiration **B1**, releasing energy for growth and reproduction **B1**; stirring spreads the nutrients, oxygen and heat evenly through the culture **B1**.
- (b) Temperature is monitored with a probe **B1** and cold water flows through the water jacket to remove the heat released by respiration **B1**; pH is monitored with a probe **B1** and a buffer, or acid or alkali, keeps it at the enzymes' optimum, as carbon dioxide from respiration lowers it **B1**.
- (c)  $\frac{95 - 36}{36} \times 100 = 163.89 \dots$  **M1** = 160% **A1**
- (d) Any four, with at least one each side. Advantages: less damage by insects, so a higher yield **B1**; less insecticide needs to be sprayed, saving money and harming fewer harmless insects **B1**. Disadvantages: the seed is expensive and new seed must be bought each year **B1**; insects may evolve resistance to the toxin **B1**; the gene could spread to wild plants in pollen; possible unknown effects on health or on food chains.

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## Common mistakes

- **Mixing up the two GM enzymes.** Restriction enzymes **cut**; DNA ligase **joins**. Examiner reports see them swapped every year, and "lipase" written for "ligase".
- **Leaving out "recombinant plasmid"**, or calling it "a GM organism". The plasmid with the gene in it is a **recombinant plasmid**; the bacterium that takes it in is the GM organism.
- **Muddled GM steps.** Sticky ends are on **DNA**, not proteins; the **whole gene** is cut out (not cut into pieces); the gene goes into a plasmid, not a plasmid into another plasmid.
- **Vague reasons for bacteria.** "They grow fast" is weaker than "they **reproduce rapidly**". Giving products (insulin, yoghurt) when asked for reasons scores nothing, and so do plasmids when the question says "apart from structural features".
- **Choosing the loop of DNA** on a bacterium diagram. The useful part is the **plasmid**, the small ring.
- **The wrong product in bread.** The dough rises because of **carbon dioxide**, not alcohol.
- **"Biofuels give off no carbon dioxide."** They do; the point is that the plants took in the same carbon dioxide while growing. And write **renewable**, not "reusable".
- **Thermometers don't control anything.** A probe **monitors** the temperature; the **water jacket** controls it.
- **Oxygen is not a nutrient.** Nutrients are glucose, amino acids and ions; name ions as ions (nitrate ions), not elements (nitrogen).
- **"Sterilise to keep it clean."** Say it **kills microorganisms** that would **compete** with the fungus or contaminate the product.

- **Answering about selective breeding or monocultures** when the question is about GM. Keep to what is special about moving genes.
- **Vague disadvantages of GM crops:** "cost" or "long-term effects" alone. Say **the seeds are expensive and must be bought every year, or the gene could spread to wild plants.**
- **Pectinase for an oil stain.** Oil needs **lipase**; pectinase is for fruit juice.

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## How to get the marks

- **"State"**: the exact term, such as restriction enzyme, DNA ligase, recombinant plasmid, pectinase, lipase, fungus, oxygen.
- **"Describe" the GM process**: one step per sentence, in order, with the key words: **restriction enzyme, sticky ends, same enzyme, complementary, ligase, recombinant plasmid, multiply, express.** A step only earns its mark in the right context.
- **"Explain"**: join each point to its reason with "so" or "because": "respiration releases heat, so cold water in the jacket keeps the temperature at the optimum".
- **"Discuss"** (Paper 4): give **both** advantages and disadvantages, each with its reason. Several different points score more than one point explained at length.
- **Word equations** earn one mark for "glucose →" and one for both products. The products can be in either order; "alcohol" is accepted for ethanol.
- **Use the mark allocation**: a 5-mark fermenter question needs at least five different points.
- **Calculations**: write the values you read, then the working, then the answer rounded as asked. The mark schemes give separate marks for the readings, the calculation and the rounding, and a later mark can still be earned after an earlier slip.
- **"Suggest"**: apply what you know to the new organism or product in the question, such as a new GM crop or a new enzyme.
- **Multiple choice**: decide each numbered statement or column on its own, then find the option that matches.

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## Quick check

1. State the two products of anaerobic respiration in yeast, and say which is used in bread-making and which as a biofuel.
2. Name the enzyme that: (a) cuts DNA to leave sticky ends; (b) joins a gene into a plasmid; (c) removes a grease stain; (d) increases the volume of fruit juice; (e) is used to make lactose-free milk.
3. Put these steps in order: P, bacteria multiply in a fermenter; Q, gene inserted into the plasmid using ligase; R, human gene cut out with a restriction enzyme; S, recombinant plasmid put into a bacterium; T, plasmid cut with the same restriction enzyme; U, bacteria make the human protein.
4. Use the washing-powder graph in "Understand it". Calculate the percentage decrease in the rate of stain removal by the biological powder from 40 °C to 60 °C, to the nearest whole number.
5. State two conditions controlled in a fermenter and how each is controlled.

## Answers

1. Ethanol (alcohol) and carbon dioxide. Carbon dioxide makes bread rise; ethanol is the biofuel.
2. (a) A restriction enzyme. (b) DNA ligase. (c) Lipase. (d) Pectinase. (e) Lactase.
3.  $R \rightarrow T \rightarrow Q \rightarrow S \rightarrow P \rightarrow U$  (R and T can be swapped: both cuts happen before the joining).
4.  $\frac{12 - 1}{12} \times 100 = 91.67$ , so a decrease of 92%.
5. Any two: temperature, by the water jacket (with a probe to monitor it); pH, by a buffer or adding acid or alkali (with a probe); oxygen, by bubbling in sterile air; nutrients, by adding sterile glucose and amino acids; waste gases, by a vent.

## Past questions to try

- [0610/43/O/N/24 Q6](#): naming the GM enzymes, why the same enzyme cuts both DNAs, stirring a fermenter, and GM crops for and against.
- [0610/42/F/M/23 Q6](#): golden rice: completing sentences about the GM steps, and the disadvantages of GM rice.
- [0610/41/O/N/24 Q6](#): penicillin in a fermenter, growth phases, air, temperature and paddles.
- [0610/32/O/N/24 Q7](#): an investigation of pectinase and the volume of fruit juice.