

Cambridge AS & A Level · Biology 9700 · Notes

Genetic technology A2

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Read first: Nucleic acids and protein synthesis, Inheritance

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What you need to know

By the end of this topic you should be able to:

1. **Define recombinant DNA.**
2. **Explain what genetic engineering is:** changing genetic material on purpose to change particular characteristics, often by moving a gene into an organism so that it is expressed.
3. **Explain the three ways to get a gene:** cut it from a donor's DNA, make it from the donor's mRNA, or make it chemically from nucleotides.
4. **Explain the roles of restriction endonucleases, DNA ligase, plasmids, DNA polymerase and reverse transcriptase** in moving a gene into an organism.
5. **Explain why a promoter may have to be transferred** with the gene.
6. **Explain how marker genes for fluorescent products** show that a gene is expressed.
7. **Explain gene editing:** inserting, deleting or replacing DNA at specific sites in the genome.
8. **Describe and explain PCR**, including the role of Taq polymerase.
9. **Describe and explain gel electrophoresis** for separating DNA fragments by length.
10. **Outline how microarrays are used** to analyse genomes and to detect mRNA in studies of gene expression.
11. **Outline the benefits of databases** of nucleotide sequences, amino acid sequences and protein structures.
12. **Explain the advantages of recombinant human proteins:** insulin, factor VIII and adenosine deaminase (ADA).
13. **Outline the advantages of genetic screening:** BRCA1 and BRCA2 (breast cancer), Huntington's disease and cystic fibrosis.
14. **Outline gene therapy** for SCID and inherited eye diseases.
15. **Discuss the social and ethical issues** of genetic screening and gene therapy.
16. **Explain how GM organisms can help feed the world:** GM salmon, herbicide-resistant soybean and insect-resistant cotton.
17. **Discuss the ethical and social implications** of GMOs in food production.

Understand it

Genetic engineering and recombinant DNA

Recombinant DNA is DNA made by **joining DNA from two different sources**, usually two species: a human gene joined to a bacterial plasmid, for example.

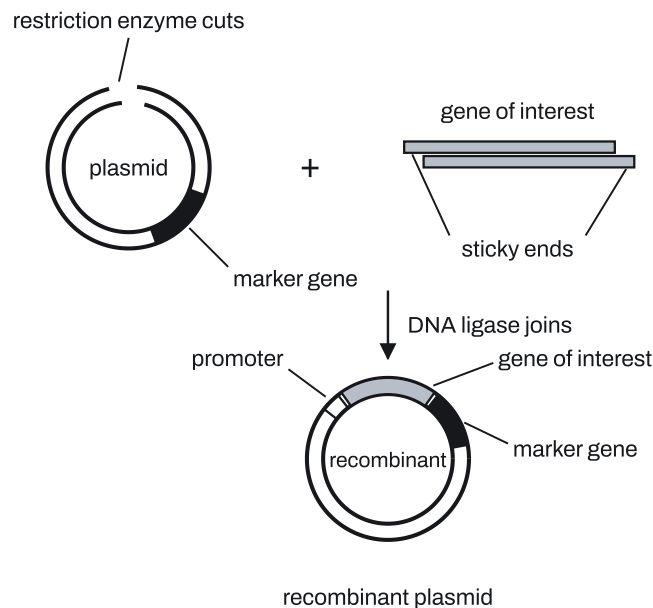
Genetic engineering is the **manipulation of genetic material**, done on purpose, so that an organism gains or loses a particular trait. Usually a gene is moved into an organism so that it is **expressed** (transcribed and translated), making its protein and changing the phenotype. The receiver is a **genetically modified organism (GMO)**, or **transgenic** organism.

Getting the gene

1. **Cut it out of the donor's DNA** with a restriction endonuclease. A eukaryotic gene from genomic DNA still has **introns** (non-coding sequences), and bacteria **cannot splice** them out, so a bacterium given it makes no working mRNA or protein.
2. **Make it from mRNA**. Extract mRNA from cells that express the gene (insulin mRNA from β -cells of the pancreas): they hold many copies, already spliced. **Reverse transcriptase** uses the mRNA as a **template** to make single-stranded **complementary DNA (cDNA)**; **DNA polymerase** makes the second strand. cDNA has **no introns**.
3. **Synthesise it chemically** by joining nucleotides in the order of a sequence from a **database**. There are no introns, and the sequence can be changed on purpose.

The enzymes and plasmids

- **Restriction endonucleases** cut DNA at a **specific recognition sequence**, breaking phosphodiester bonds. Many make a staggered cut, leaving short single-stranded **sticky ends**. Cutting the gene and the plasmid with the **same** enzyme gives **complementary** sticky ends.
- **DNA ligase** joins the gene to the plasmid: the sticky ends pair by **hydrogen bonds**, and ligase forms **phosphodiester bonds** that seal the **sugar-phosphate backbones**.
- **DNA polymerase** adds free DNA nucleotides to a growing strand, pairing them with a template. It makes cDNA double-stranded, and copies DNA in PCR.
- **Reverse transcriptase** makes cDNA using mRNA as a template.
- **Plasmids** are small circles of double-stranded DNA in bacteria, separate from the chromosome, that copy themselves. They are the usual **vector**, carrying the gene into the host. Bacteria take up plasmids after calcium ions and a **heat shock**, and pass them to other bacteria (conjugation). Other vectors are **viruses** and **liposomes**; eggs can be **microinjected** and plant cells hit with a **gene gun**.



Promoters

A **promoter** is the DNA just before a gene where **RNA polymerase** and **transcription factors** bind, so that **transcription** starts. A bacterium's RNA polymerase does not recognise a human promoter, so a promoter that works in the host is put in front of the gene, facing the right way (a gene inserted backwards is not transcribed into the right mRNA). A promoter can also control **where and when** the gene is expressed, such as only in seeds, or all year.

Marker genes

Only some cells take up the plasmid, and not every plasmid has the gene. A gene for a **fluorescent protein** (such as GFP, from a jellyfish) is placed **next to the gene of interest, after the same promoter**, so both are expressed together. Cells that glow under **UV or blue light** were **transformed** and are **expressing** the gene of interest. The protein is harmless, and cells are checked without killing them. Older methods used **antibiotic-resistance** genes: only bacteria with the plasmid grow on that antibiotic.

Gene editing

Gene editing inserts, deletes or replaces DNA at a specific site in an organism's own genome. In **CRISPR/Cas9**, a short **guide RNA** with bases **complementary** to the target DNA is joined to **Cas9**, a nuclease. The guide RNA finds the target by base pairing and Cas9 cuts both strands there. The cell's repair can delete bases (knocking the gene out) or, with a DNA template supplied, insert or replace a sequence. Changing one triplet changes one codon, so one amino acid.

	Transfer using a plasmid	Gene editing
What changes	a whole gene added, often from another species	bases at one chosen site in the organism's own DNA

	Transfer using a plasmid	Gene editing
Tools	restriction enzymes, ligase, vector, marker genes	guide RNA and a nuclease (Cas9)
Result	a transgenic organism	often no foreign DNA, like a natural mutation; inserting another species' DNA makes it transgenic

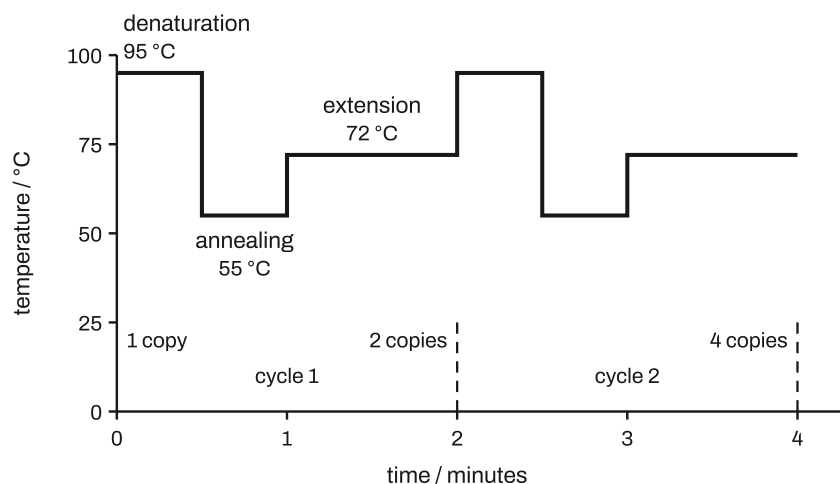
Both can make an organism produce a different protein, or stop producing one.

PCR

The **polymerase chain reaction (PCR)** makes millions of copies of one piece of DNA (it **amplifies** it). The tube holds the DNA; two **primers** (short single strands of DNA complementary to the two ends of the target); free **DNA nucleotides**; **Taq polymerase**; and a **buffer** for the pH. A machine cycles the temperature:

1. **Denaturation, 90–98 °C** (about 95 °C): **hydrogen bonds** between bases break and the strands **separate**.
2. **Annealing, 50–65 °C** (about 55 °C): the **primers bind** to complementary bases at the ends of the target on each strand, giving Taq polymerase a **starting point**.
3. **Extension, 68–75 °C** (about 72 °C): **Taq polymerase** adds nucleotides to each primer by complementary base pairing, building a **new complementary strand**.

Each cycle **doubles** the DNA, so after n cycles one molecule becomes 2^n .

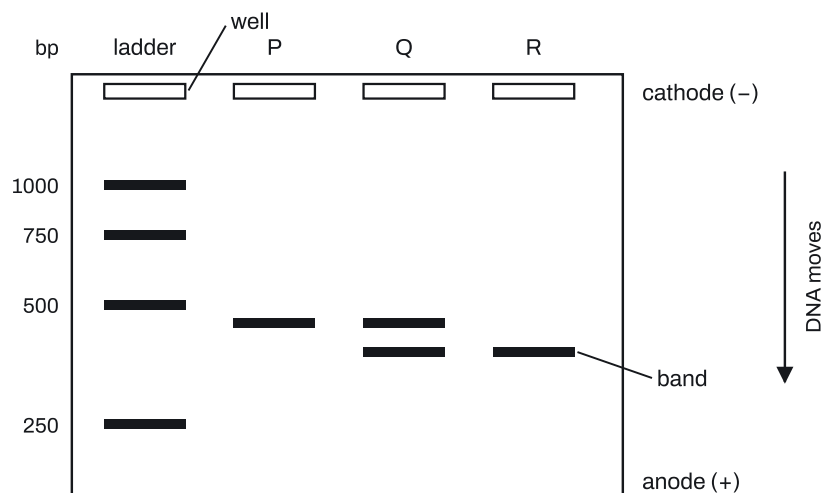


Taq polymerase is a DNA polymerase from *Thermus aquaticus*, a hot-spring bacterium. It is **thermostable**: **not denatured at 95 °C**, with an optimum of about 72 °C, so it lasts through every cycle and is not replaced.

PCR is used when there is **too little DNA** (a drop of blood, a crime scene, water samples), to pick out **one fragment** small enough to sequence, and to test for alleles.

Gel electrophoresis

1. DNA samples go into **wells** at one end of an agarose **gel** in buffer; a **DNA ladder** (fragments of known lengths) goes in another lane.
2. A voltage is applied. DNA is **negatively charged** (its phosphate groups), so it moves towards the **anode** (+).
3. The gel is a mesh: **shorter fragments move faster**, so they go **further** in the same time.
4. Fragments of one length form a **band**, stained or fluorescent under UV light. Comparing with the ladder gives their lengths.



It checks that PCR worked, tells a normal allele from a mutant one of different length, and compares people's band patterns (DNA profiling). Paper chromatography, by contrast, separates pigments by solubility in a solvent and identifies them by R_f values.

Microarrays

A **microarray** is a small chip holding thousands of different **single-stranded DNA probes**, each in a known position and complementary to one gene or sequence.

- **Analysing a genome** (which sequences are present or deleted): cut the DNA into fragments, separate the strands and label them with a **fluorescent tag**. They **hybridise** (complementary base pairing) to matching probes; unbound DNA is washed off and the chip scanned. A glowing spot means that sequence is present; its brightness shows how many copies. Two genomes in two colours can be compared.
- **Studying gene expression** (which genes are switched on): extract **mRNA**, use **reverse transcriptase** to make labelled **cDNA**, and hybridise it. A **brighter** spot means **more mRNA**, so the gene is **expressed more**.

Databases and bioinformatics

Online **databases** store **nucleotide sequences** of genes and genomes, **amino acid sequences** and **protein structures**. **Bioinformatics** uses computers to store, search and analyse them. The benefits: data are **shared** quickly worldwide; a new sequence can be **compared** with thousands of others (similar genes, **evolutionary relationships**, identifying species); a gene's sequence can be found to **synthesise it** or design **primers**; a protein's **structure and function** can be predicted; **mutations** linked to disease found; and drug or vaccine targets identified (for example in the malaria parasite, *Plasmodium*).

Recombinant human proteins

GM bacteria or yeast make human **insulin** (for diabetes; once from pigs and cattle), **factor VIII** (a clotting protein for **haemophilia**; once from donated blood) and **ADA** (for **SCID**, given by regular injection instead of a bone marrow transplant).

The advantages: **identical to the human protein**, so **fewer immune or allergic reactions**; a **large, reliable supply** that matches demand; **no risk of infection** (such as HIV from donated blood); **no ethical or religious objections** to animal sources; pure and consistent; and, for ADA, **no donor needed**. **Yeast** is sometimes the host: a eukaryote with a Golgi body to modify proteins as human cells do, cheap to grow, and able to secrete the protein, which is easier to purify.

Genetic screening

Genetic screening tests DNA for alleles that cause, or raise the risk of, a disease.

- **BRCA1 and BRCA2** are tumour-suppressor genes whose proteins repair DNA. A faulty allele gives a non-functional protein, so mutations build up and cells may divide uncontrollably: breast cancer is much more likely. People with a **family history** are tested. A positive result allows **frequent checks, early treatment**, lifestyle changes or **preventive surgery**, and informed decisions about children; a negative result ends the worry.
- **Huntington's disease**: a **dominant** allele, symptoms in middle age, no cure. Testing lets people plan and decide about having children.
- **Cystic fibrosis**: **recessive** alleles of the CFTR gene. Carrier testing tells couples their risk; testing newborns lets treatment start early.

Gene therapy

Gene therapy adds a **normal allele** to a person's cells to treat a genetic disease. The faulty allele is **not removed**, so it works for **recessive** conditions. Only body cells are treated, so it is not inherited.

- **SCID (ADA deficiency)**. Without ADA, toxic substances kill lymphocytes. **Bone marrow stem cells** are removed; a **viral vector** (a retrovirus) inserts the normal ADA allele into their DNA; a drug clears space in the bone marrow; the cells are **infused** back and make lymphocytes that produce ADA. Stem cells last, so one treatment can be enough. In early trials some patients developed **leukaemia** where the vector inserted.

- **Inherited eye diseases** (such as one caused by a faulty RPE65 gene, where retinal cells die): the normal allele, in a harmless **adeno-associated virus (AAV)**, is **injected into the eye**; retinal cells make the working protein. The eye is **easy to reach**, small (so **little vector**), has a **low immune response**, and the other eye is a comparison.

Adding a normal allele cannot mask a **dominant** allele such as Huntington's; that needs gene editing.

Social and ethical issues in medicine

- **For:** early diagnosis and treatment; better quality of life and life expectancy; informed choices about children; one treatment instead of lifelong injections.
- **Against:** high cost and unequal access; inaccurate results; anxiety about an incurable disease; **consent** (newborns) and **privacy** of genetic data (insurers, employers); pressure to end pregnancies; unknown long-term effects; **cancer** or immune reactions from vectors; fears of "designer babies"; religious objections.

GM organisms in agriculture

More food is needed from the same land. Genetic engineering raises the **quality** and **productivity** of crops and animals, faster than selective breeding (one generation, not many).

- **GM salmon:** a growth hormone gene with a promoter that keeps it switched on all year, so the fish reach market size in about half the time on less food. They are sterile females farmed in tanks on land, so they cannot breed with wild salmon.
- **Herbicide-resistant soybean:** a bacterial gene for an enzyme the herbicide does not inhibit. Spraying kills the **weeds but not the crop**, so there is **less competition** for light, water and minerals and a **higher yield**.
- **Insect-resistant (Bt) cotton:** a gene from *Bacillus thuringiensis* for **Bt toxin**, which kills insect larvae that eat the plant but not mammals. **Less damage, higher yield and less insecticide**.

Ethical and social implications of GM food

- **Benefits:** more food; cheaper food; better nutrition; less pesticide; less land needed, leaving more for wildlife; more income for farmers.
- **Concerns:** possible **allergies**; **herbicide residue** on food; **resistant weeds and pests**; **pollen** carrying genes to non-GM, organic or wild plants; GM crops becoming weeds; lower biodiversity; costly **patented** seed bought each year from a few companies; **consumer mistrust** and **labelling**. Some countries **ban** growing GM crops but import GM food.

Key facts and formulas

No formula is given on the paper for this topic. "Learn it" means you must remember it.

Formula	Status
Copies after PCR = $N_0 \times 2^n$, for N_0 starting copies and n cycles	Learn it
Reaching the same amount k cycles later means 2^k times less DNA at the start	Learn it
Part of a total = $\text{total} \times \frac{\text{percentage}}{100}$	Learn it
Percentage change = $\frac{\text{new} - \text{original}}{\text{original}} \times 100$	Learn it

Also learn the PCR temperatures (denaturation 90–98 °C, annealing 50–65 °C, extension 68–75 °C) and a one-line role for each enzyme.

How to do it

We have tagged 53 Paper 4 questions for this topic, 2021–2025 (45 different: 8 are repeated on Paper 41). 42 of the 53 mix types, so the counts add up to more than 53.

Question type	Questions
Interpret data from a genetic technology study	18
Discuss social and ethical issues	17
Outline how a gene is transferred	12
Describe and explain PCR	11
Gene editing	10
Name or give the roles of the enzymes	10
How a GM crop or animal helps meet food demand	9
Advantages of recombinant human proteins	8
Databases and bioinformatics	8
Why a promoter is transferred	6
Gene therapy	6
Calculate from data	6
Gel electrophoresis	6
Ways to obtain a gene	5
Marker genes	5
Define recombinant DNA or genetic engineering	5
Microarrays	4
Advantages of genetic screening	2

1. Interpret data from a genetic technology study (18 questions)

1. Read the axes, units, key, and which group is treated and which is the control.
2. **Describe**: the trend, how the groups differ, and a **data quote** (paired values with units and the time).
3. Point out anything that breaks the pattern (an anomaly).
4. **Does it support the hypothesis?** Points for (with data), then against: no statistical test, small sample, short study.

Variations: why a control is used (a **baseline** for comparison); which plant is the wild type (the one without the new trait); why two GM varieties were made (to see which gene works better); which quantitative PCR curve started with most DNA (the one crossing the threshold in fewest cycles).

2. Discuss social and ethical issues (17 questions)

Use the lists in "Understand it", both sides, each point tied to the stem. "Social" means effects on people (food, cost, health); "ethical" means right and wrong (consent, access). If only **benefits** are asked for, give only benefits.

Variations: why a GM crop is eaten in many countries but grown in few (unsuitable climate or soil, no approval or a ban, opposition, no spare land); screening newborns for a rare disease that has an expensive gene therapy (no family history needed, early treatment, family planning, no extra sample, but cost, accuracy, unknown long-term effects); privacy of DNA databases.

3. Outline how a gene is transferred (12 questions)

1. Obtain the gene (restriction enzyme, reverse transcriptase, or synthesis); PCR can copy it.
2. Cut the plasmid with the **same restriction enzyme**, giving complementary **sticky ends**.
3. The sticky ends pair; **DNA ligase** forms phosphodiester bonds.
4. Add a **promoter** so the gene is expressed, and a **marker gene**.
5. Put the vector into the host (heat shock for bacteria; microinjection or a virus for eggs; a gene gun for plant cells).
6. Pick out transformed cells with the marker; grow them and purify the product.

Variations: joining two sequences into one gene first (ligase); how plasmids spread between bacteria (conjugation, transformation); how an introduced protein blocks other genes (it stops transcription factors or RNA polymerase binding to their promoters).

4. Describe and explain PCR (11 questions)

Give each step with its **temperature**, **what happens** and **why**, then "repeated; each cycle doubles the DNA".

Variations: substances in the tube, each with a reason (nucleotides pair with the template; Taq builds new strands and is thermostable; buffer keeps the pH); the role of Taq alone (DNA polymerase, binds at the primers, extends them, lasts all the cycles, optimum 72 °C); why PCR comes before a test (too little DNA;

fragments short enough to sequence); how PCR, electrophoresis and sequencing compare two populations' DNA.

5. Gene editing (10 questions)

Use **insert, delete or replace, specific site** and **own DNA**; for CRISPR/Cas9, the guide RNA is **complementary** to the target and Cas9 **cuts**. Link to the outcome: the faulty sequence is removed or corrected, so a functional protein is made, or a gene is no longer expressed.

Variations: tick tables against plasmid transfer (use the table above); why only one gene was affected (only it matches the guide); changing a codon to code for a different amino acid (insulin analogues); why editing suits a dominant allele.

6. Name or give the roles of the enzymes (10 questions)

Restriction endonuclease **cuts** at a specific sequence; ligase forms **phosphodiester bonds**, joining sugar-phosphate backbones and adding a gene to a plasmid; DNA polymerase **adds free nucleotides** to make a complementary strand; reverse transcriptase uses **mRNA as a template** to make cDNA. **Variation:** their natural roles (cutting viral DNA in bacteria; joining DNA in replication; copying DNA; making DNA from a retrovirus's RNA).

7. How a GM crop or animal helps meet food demand (9 questions)

Build the chain: **new trait** → **less loss or faster growth** → **higher yield**. Herbicide resistance: weeds killed, not the crop → less competition for light, water and minerals. **Variations:** why genetic engineering is faster than selective breeding; features of dairy cattle to improve (milk yield and quality).

8. Advantages of recombinant human proteins (8 questions)

Compare with the old source, with specific points ("no risk of HIV from donated blood"), not "it is easily available". **Variations:** yeast as the host; a 7-mark essay on genetic technology in medicine (the three proteins and their diseases, screening, gene therapy).

9. Databases and bioinformatics (8 questions)

Give different benefits from the list above. **Variation:** a 6-mark essay on bioinformatics after sequencing the human and *Plasmodium* genomes.

10. Why a promoter is transferred (6 questions)

RNA polymerase and transcription factors **bind** to it, so the gene is **transcribed**; the host may not recognise the donor's promoter. **Variation:** diagrams of plasmids: the protein is made only if the gene is the right way round after the promoter; a backwards gene or empty plasmid makes none.

11. Gene therapy (6 questions)

The steps in order: normal allele in a vector → into the patient's cells → allele expressed → protein made.

Variations: why the eye suits it; why a large gene will not fit a small vector; why antibodies against a vector stop it working.

12. Calculate from data (6 questions)

1. PCR: copies = $N_0 \times 2^n$, in standard form if asked.
2. Percentages: one step per line. If GM maize grew on 64% of 40 million hectares and maize was 32% of all GM crops: $0.64 \times 40 = 25.6$ million ha, and $25.6 \div 0.32 = 80$ million ha of GM crops.
3. Quantitative PCR: k cycles apart means 2^k times different amounts; 4 cycles apart is 16 times.

13. Gel electrophoresis (6 questions)

DNA **negative** → moves to the **anode**; **shorter fragments move further**; the **ladder** gives lengths and shows the gel ran properly. The **shorter** allele's band is **further** from the well; **two bands** means both alleles. Check who counts as affected: if one mutant copy matters, heterozygotes are affected too.

14. Ways to obtain a gene (5 questions)

The three routes, each with its enzyme. **Variation:** why cDNA is expressed in bacteria but genomic DNA is not (introns; bacteria cannot splice).

15. Marker genes (5 questions)

Next to the gene of interest, same promoter, both expressed; **glows under UV light**; shows cells are **transformed** and **express** the gene. **Variation:** antibiotic-resistance markers.

16. Define recombinant DNA or genetic engineering (5 questions)

Recombinant DNA: **DNA from two different sources joined**. Genetic engineering: **deliberate manipulation of genetic material**, often transferring a gene so it is **expressed**.

17. Microarrays (4 questions)

Decide first: **genome** (DNA: is a sequence present?) or **expression** (mRNA → cDNA: how much?). Then: labelled single strands **hybridise** to probes, wash, scan; brightness = amount. **Variation:** half the normal fluorescence means one of the two copies is deleted.

18. Advantages of genetic screening (2 questions)

Why test (family history); what a positive result allows (checks, early treatment, surgery, family planning); a negative result gives relief. **Variation:** why a faulty BRCA1 protein raises cancer risk (DNA not repaired, mutations build up, uncontrolled division).

Worked examples

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.
-

Example 1

A company plans to use bacteria to make human growth hormone.

(a) Explain why the gene is made from mRNA taken from pituitary cells, rather than cut out of human genomic DNA. **[3]**

(b) Outline how the gene is inserted into a plasmid, and how bacteria that express the gene could be identified. **[5]**

(a) Any three, **B1** each: genomic DNA contains introns (non-coding); bacteria cannot remove introns (no splicing); so no functional mRNA or protein would be made; reverse transcriptase makes cDNA from the already-spliced mRNA, so it has no introns; pituitary cells hold many copies of this mRNA.

(b) Any five, **B1** each: cut the plasmid with the same restriction enzyme as the gene; giving complementary sticky ends; which pair by hydrogen bonds; DNA ligase forms phosphodiester bonds; a promoter is added in front of the gene; a fluorescent marker gene is inserted next to it, after the same promoter; bacteria that glow under UV light have the plasmid and express the gene.

Example 2

An allele that causes a disease has a deletion of 70 base pairs. PCR copies one region of the gene: 450 base pairs from the normal allele, 380 from the mutant allele. The products from people P, Q and R are separated as in the gel diagram in "Understand it".

(a) Describe the steps of one PCR cycle. **[3]**

(b) Explain how gel electrophoresis separates the PCR products. **[3]**

(c) State which people have the mutant allele. **[1]**

(d) Sequencing needs at least 5×10^8 copies. The PCR starts with 4 copies. Calculate the smallest number of cycles needed. **[2]**

(a) 95 °C: hydrogen bonds break and the strands separate **B1**. 55 °C: primers anneal to complementary bases at each end of the target **B1**. 72 °C: Taq polymerase adds nucleotides to the primers, making new strands **B1**.

(b) Any three, **B1** each: DNA is negatively charged; so it moves through the gel to the anode; shorter fragments move faster, so further; the 380 bp product goes further than the 450 bp product; the ladder confirms the lengths.

(c) Q and R (Q has both bands: heterozygous). **B1**

(d) $4 \times 2^n \geq 5 \times 10^8$, so $2^n \geq 1.25 \times 10^8$. **M1** $2^{26} = 67\,108\,864$ is too few and $2^{27} = 134\,217\,728$ is enough: **27 cycles. A1**

Example 3

Farmers compared insect-resistant (Bt) cotton with non-GM cotton.

	yield / kg per hectare	insecticide sprays per season
non-GM cotton	1850	9
Bt cotton	2350	3

(a) Calculate the percentage increase in yield of the Bt cotton. **[2]**

(b) Explain how the Bt gene leads to these results. **[3]**

(c) Discuss the social implications of growing Bt cotton. **[4]**

(a) $\frac{2350 - 1850}{1850} \times 100$ **M1** = 27.0% **A1**

(b) The Bt gene codes for a toxin that kills insect larvae eating the plant **B1**; less of the crop is damaged, so the yield is higher **B1**; the plant protects itself, so fewer sprays are needed **B1**.

(c) Any four, both sides, **B1** each: higher yield and income; lower costs, as less insecticide is bought; farmworkers less exposed to insecticide; but GM seed is costly and bought each year; insects may evolve resistance to the toxin; pollen may carry the gene to non-GM crops; some consumers mistrust GM products.

Example 4

SCID can be caused by recessive alleles of the gene for ADA. Huntington's disease is caused by a dominant allele.

(a) Outline how gene therapy is used to treat SCID. **[4]**

(b) Explain why adding a normal allele would not treat Huntington's disease, and suggest how gene editing could. [3]

(a) Any four, **B1** each: bone marrow stem cells are removed; a viral vector carries a normal ADA allele into them; it is inserted into their DNA; a drug clears space in the bone marrow; the cells are infused back; they make lymphocytes that produce functional ADA.

(b) The dominant allele is still expressed, so its harmful protein is still made **B1**. A guide RNA complementary to the allele could direct Cas9 to cut there **B1**, deleting or replacing the faulty sequence at that specific site, so the harmful protein is no longer made **B1**.

Common mistakes

- **Reverse transcriptase "converts" mRNA to DNA.** It uses mRNA as a **template** to make cDNA.
- **Wrong enzyme names:** ligase, not helicase, joins DNA; say **DNA** polymerase, not "polymerase".
- **Taq polymerase "transcribes".** It makes DNA. Give its role and why heat stability matters (it survives 95 °C every cycle).
- **Gene therapy "replaces" the faulty allele.** It **adds** a normal one.
- **Mixing up microarray uses:** genomes use DNA; expression uses mRNA made into cDNA. Microarrays don't tell individuals apart: use electrophoresis or sequencing.
- **Putting mRNA into the plasmid,** or the plasmid into seeds rather than cells.
- **Vague advantages** such as "easily available": say what is better.
- **Data answers** that only list numbers, without units, the matching time or a conclusion.
- **Herbicide-resistant crops need less herbicide:** not so; they let the farmer spray it.

How to get the marks

- **"Outline"** and **"describe"** a process: steps in order, each with its key word (sticky ends, phosphodiester bonds, promoter, primers anneal); marked "any four (or five) from" a list.
- **"Explain"** needs the reason: "primers bind **to give Taq a starting point**".
- **PCR:** three temperatures, what happens, and "repeated".
- **"Discuss":** both sides, tied to the context. **"Suggest":** apply what you know to the new example.
- **Data:** trend, comparison, anomaly and a data quote with units.
- **Calculations:** show working; standard form and significant figures as asked.
- **Enzymes in full:** "restriction endonuclease", "DNA ligase", "reverse transcriptase", "Taq polymerase".

Quick check

1. Name the enzyme that (a) makes cDNA from mRNA; (b) forms phosphodiester bonds between a gene and a plasmid; (c) cuts a plasmid at a specific sequence; (d) extends primers in PCR.

2. After 10 cycles of PCR a tube holds 30 720 copies of a DNA fragment. How many copies were there at the start?
3. Explain why a fluorescent marker gene is placed next to the gene of interest, after the same promoter.
4. In a country, 1 in 2500 newborn babies has cystic fibrosis. Estimate how many of 48 000 babies born in a year have it, and give two advantages of screening newborns for it.
5. mRNA from a tumour and from normal tissue is compared on one microarray. Outline what is done with the mRNA, and state what a spot much brighter for the tumour sample shows.

Answers

1. (a) Reverse transcriptase. (b) DNA ligase. (c) Restriction endonuclease. (d) Taq polymerase.
2. Each cycle doubles the DNA: $30\,720 \div 2^{10} = 30\,720 \div 1024 = 30$ copies.
3. Both genes are transcribed together, so cells that glow under UV light have taken up the gene **and** are expressing it; they are found without harming them.
4. $48\,000 \div 2500 = 19.2$, so about 19 babies. Any two: treatment starts early; better quality of life and life expectancy; parents learn their risk for future children; it uses a blood sample already taken.
5. Reverse transcriptase makes cDNA from each sample; each is labelled with a different fluorescent dye; both hybridise to the probes by complementary base pairing; unbound cDNA is washed off and the chip scanned. A brighter tumour spot means more mRNA of that gene: it is expressed more in the tumour.

Past questions to try

- [9700/43/O/N/24 Q8](#): making a gene, enzyme roles and the PCR temperatures.
- [9700/43/M/J/24 Q4](#): enzyme roles, the promoter, and which plasmids make insulin.
- [9700/42/O/N/23 Q4](#): fluorescent marker genes and microarrays.
- [9700/43/M/J/23 Q4](#): gene therapy for SCID and its ethical issues.