

Gene technology

Revision notes · Making recombinant DNA, inserting it into cells, drugs from GMOs, microarrays, bioinformatics and the risks and benefits of GMOs.

For Pearson Edexcel International AS Biology XBI11 and International A Level Biology YBI11. **A Level only** is IA2 content (Units 4 to 6), not needed for the International AS.

Gene technology **Spec 8.17-8.22** **A Level only**

Making recombinant DNA **Spec 8.18**

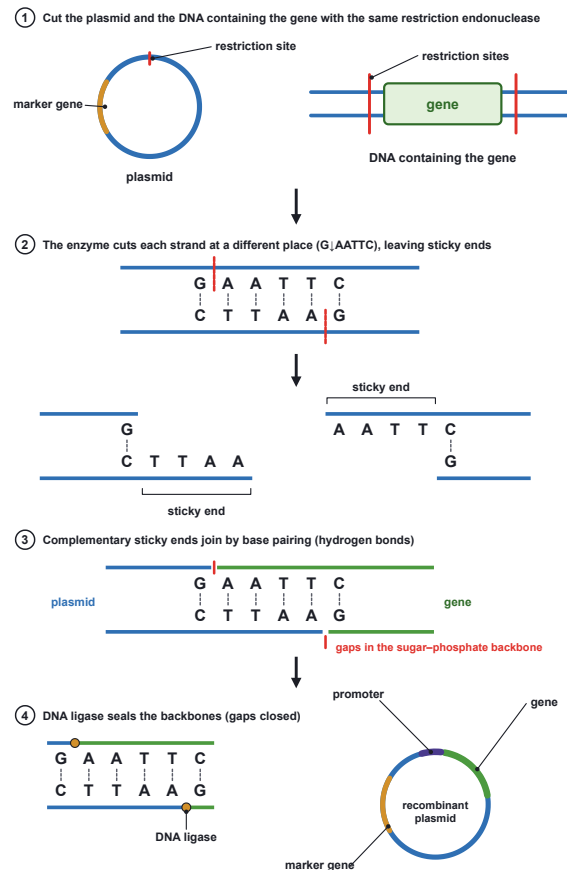
Recombinant DNA is DNA made by joining DNA from two different sources, usually from different organisms. A gene from one organism is combined with a **vector**, usually a **plasmid** (a small circular DNA molecule from bacteria), which carries the gene into a host cell.

Getting the gene

- **Using reverse transcriptase**: mRNA for the gene is extracted from cells that make a lot of it (for example, insulin mRNA from pancreatic beta cells). Reverse transcriptase makes a complementary DNA (**cDNA**) copy, and DNA polymerase makes it double-stranded. cDNA has no introns, so a bacterium can express it.
- **Using restriction endonucleases** to cut the gene out of the genome.
- **Synthesising** the gene from its known base sequence in a gene machine.

Joining the gene to the plasmid

1. A **restriction endonuclease** (restriction enzyme) cuts DNA at a specific base sequence called its **recognition site** (often a palindromic sequence). It hydrolyses the phosphodiester bonds in each strand.
2. Many restriction enzymes make staggered cuts, leaving short single-stranded ends called **sticky ends**.
3. The plasmid is cut with the **same** restriction enzyme, so its sticky ends are complementary to those on the gene.
4. The gene and plasmid are mixed. The sticky ends join by hydrogen bonds between complementary bases (annealing).
5. **DNA ligase** forms phosphodiester bonds between the sugar and phosphate groups of adjacent nucleotides (a condensation reaction), sealing the gaps in the backbone to make a **recombinant plasmid**.



Making a **recombinant plasmid** with a restriction endonuclease and DNA ligase.

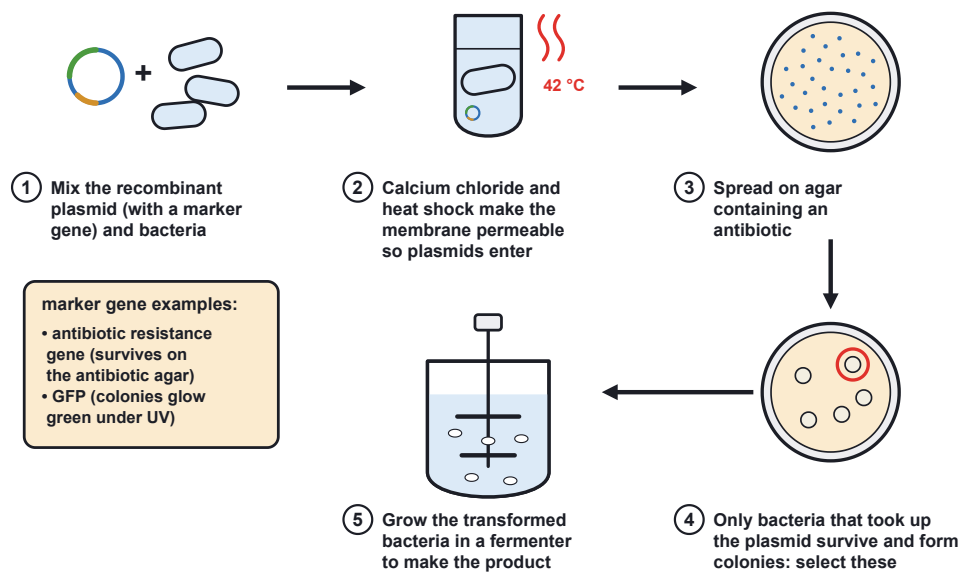
Common mistake: Restriction enzymes and ligase have opposite roles: restriction enzymes cut (hydrolysis) and ligase joins (condensation). The sticky ends are held by hydrogen bonds between bases; ligase makes the covalent bonds.

Inserting recombinant DNA into cells Spec 8.19

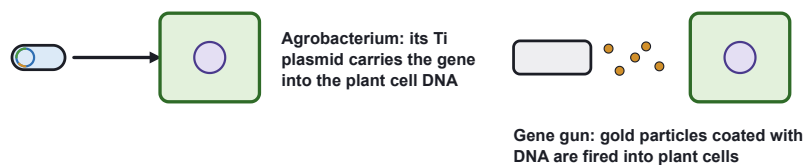
Ways of inserting recombinant DNA		
Host	Method	How it works
Bacteria	Heat shock with calcium chloride; electroporation	Cells are treated with ice-cold calcium chloride and heated briefly to about 42 °C, which makes the cell membrane permeable to plasmids. Electroporation uses a brief electrical pulse to the same effect.
Plants	Agrobacterium tumefaciens	The gene is put into the Ti plasmid of this bacterium, which naturally transfers part of its plasmid DNA into plant cells, where it is integrated into the plant genome. Whole plants are grown from the transformed cells by tissue culture.
Plants	Gene gun	Microscopic gold or tungsten particles coated with the DNA are fired into plant cells.
Animals	Microinjection	DNA is injected through a fine needle into the nucleus of a fertilised egg, which is then implanted in a surrogate mother.
Animals / human cells	Viral vectors or liposomes	A virus modified so it cannot cause disease carries the gene into cells; liposomes are lipid vesicles that fuse with the cell surface membrane.

Only a small fraction of host cells take up the DNA. **Marker genes** are included in the plasmid so that cells that took it up can be identified. These may code for antibiotic resistance (cells that survive on agar

containing the antibiotic carry the plasmid) or for a fluorescent protein such as GFP (cells glow green under UV light).



Getting a gene into plant cells



From recombinant plasmid to **transgenic bacteria**, selected using a marker gene.

Exam tip: Say why a marker gene is needed: not every cell takes up the plasmid, so a marker lets you pick out the ones that did.

Drugs from genetically modified organisms Spec 8.17

Making drugs with GMOs		
Organism	How it is used	Example
Microorganisms (bacteria, yeast)	The human gene is inserted on a plasmid; the cells are grown in large fermenters and the protein is purified from the culture	Human insulin for diabetes; human growth hormone
Plants	A human gene is inserted into plant cells, and the protein is extracted from leaves or seeds	Antibodies and vaccine antigens made in tobacco or other plant leaves
Animals	The gene is joined to a promoter that works in the mammary gland, so the protein is secreted in milk	Human antithrombin (a protein that prevents unwanted blood clotting) in the milk of transgenic goats; human clotting factor IX in the milk of transgenic sheep

Advantages of making human insulin in GM microorganisms rather than extracting it from animals:

- The insulin is identical to human insulin, so it is less likely to cause an immune response.
- It can be made in large amounts, quickly and cheaply, in fermenters.

- There are fewer ethical objections than using animals, and a reliable supply that does not depend on slaughter of animals.

Bacteria cannot remove introns and cannot modify proteins after translation in the way eukaryotic cells do (for example by adding carbohydrate groups). So a human gene for bacteria is supplied as cDNA, and proteins that need these modifications to work are made in yeast, plants or animals instead.

Exam tip: A 'how are drugs produced using GMOs' answer needs the sequence: isolate the human gene, insert it into a vector, put the vector in the host, express the gene and purify the protein.

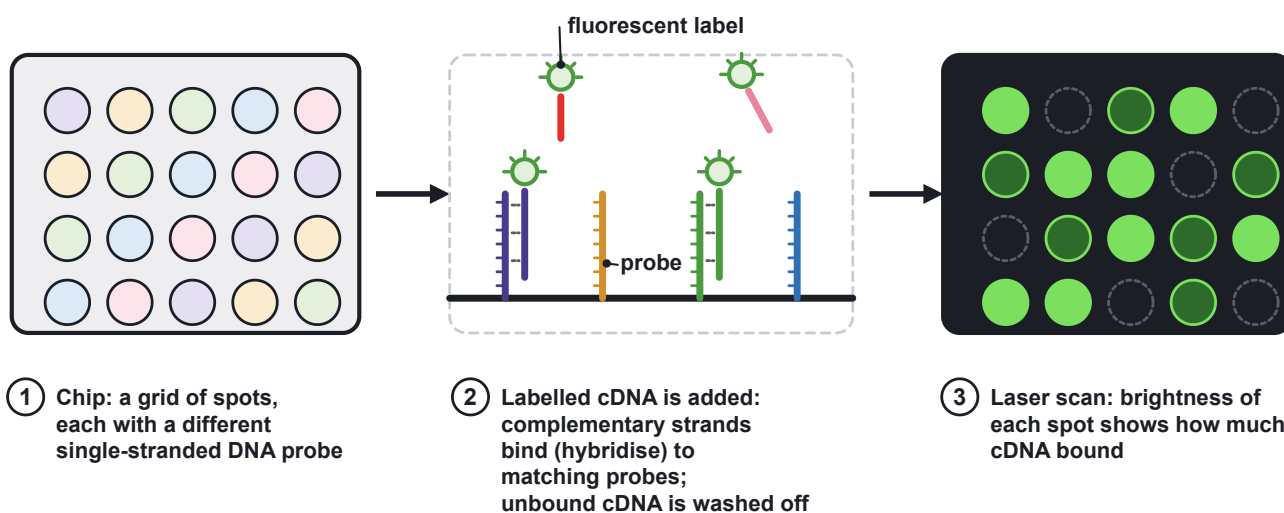
Microarrays and bioinformatics Spec 8.20, 8.21

Microarrays

A **microarray** (DNA chip) is a small slide with thousands of tiny spots in a grid. Each spot holds many copies of a single-stranded DNA **probe** from a known gene. It is used to find out which genes are active (being transcribed) in a tissue at a given time.

1. mRNA is extracted from the cells being studied (for example cancer cells and normal cells).
2. Reverse transcriptase makes cDNA from the mRNA, and a fluorescent label is added. Samples from two conditions may carry different colours.
3. The labelled cDNA is added to the microarray. It binds (hybridises) to the probes with a complementary base sequence.
4. Unbound cDNA is washed away and the array is scanned. A spot that fluoresces shows the gene was active; the brighter the spot, the more mRNA was present.

Microarrays can identify active genes in different tissues, compare healthy and diseased cells, and show how gene expression changes in response to a drug.



Key to the scan

- bright spot: a lot of cDNA bound, so the gene is highly expressed in the sample
- dull spot: a little cDNA bound, so the gene is expressed at a low level
- no spot: no cDNA bound, so the gene is not expressed in the sample

A **microarray** shows which genes are being expressed.

Bioinformatics

Bioinformatics is the use of computer science, mathematics and statistics to collect, store, analyse and interpret large sets of biological data, such as DNA base sequences, protein sequences and gene

expression data. Computers can compare sequences in databases, find genes in a genome and compare species, and so provide a way to handle the very large amount of data produced by sequencing and microarrays.

Exam tip: Define bioinformatics in terms of computers and biological data. Examples help: comparing a gene sequence in a database to identify its function.

Risks and benefits of GMOs Spec 8.22

Risks and benefits of using GMOs		
	Benefits	Risks
Agriculture	Higher yields; crops resistant to pests (such as Bt crops), herbicides, drought or disease; less pesticide used; crops with added nutrients, such as beta-carotene (which the body converts to vitamin A) in golden rice	Genes may spread to wild relatives or weeds through pollen; pests may evolve resistance; non-target insects may be harmed; reduced biodiversity if only a few GM varieties are grown
Medicine	Large amounts of pure human proteins such as insulin; vaccines and antibodies made in plants and animals	Unforeseen effects of inserted genes; welfare of transgenic animals
Health	Foods may be more nutritious or free of allergens	Possible allergic reactions to new proteins; long-term effects not fully known
Economic and ethical	Cheaper food and reliable supply; benefits for food security	Seeds are patented and costly for farmers; some people object on ethical or religious grounds to modifying organisms

In an 'evaluate' or 'discuss' question, weigh the benefits against the risks, give specific examples and reach a justified judgement. The evidence often depends on the organism, the gene and the environment, so each GMO may be judged separately.

Common mistake: Do not write that GM food 'is unsafe' or 'is safe' without evidence. Say what could happen and how it can be tested or controlled, for example by trials and monitoring.

Quick check

1. What is the role of a restriction endonuclease?
2. What does DNA ligase do?
3. Why is a marker gene included in a plasmid?
4. What does a bright spot on a microarray show?
5. What is bioinformatics?

Quick check answers

Gene technology

1. It cuts DNA at a specific recognition sequence, leaving sticky ends.
2. It joins sugar-phosphate backbones of DNA fragments by forming phosphodiester bonds.
3. To identify the host cells that have taken up the plasmid.
4. That the gene with the matching probe is active, because mRNA from it was present.
5. The use of computers and statistics to store and analyse large sets of biological data.