

Genetic technology

A-Level Biology 9700 · Topic 19 revision notes

Everything from the 5 lessons of this topic on a few pages: the key idea of each lesson, the diagram to remember, the points to learn and the traps examiners set. Read it, cover it, test yourself.

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|---------------------------------------|--|
| 1. <u>Recombinant DNA</u> | 3. <u>Microarrays and bioinformatics</u> |
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1 Recombinant DNA

Syllabus 19.1

Recombinant DNA is DNA made by joining together DNA from two different sources, often from different species. **Genetic engineering** is the deliberate manipulation of genetic material to modify specific characteristics of an organism; often a gene is transferred into another organism so that it is **expressed** there. The gene can be **extracted from donor DNA** with restriction endonucleases, **made from mRNA** with reverse transcriptase and DNA polymerase, or **synthesised chemically** from nucleotides. A **plasmid** cut with the same restriction endonuclease has complementary sticky ends; **DNA ligase** seals the gene into it. A **promoter** that the host's RNA polymerase recognises is transferred with the gene so that it is transcribed, and a **marker gene** coding for a fluorescent protein such as GFP shows which cells are expressing the new DNA.

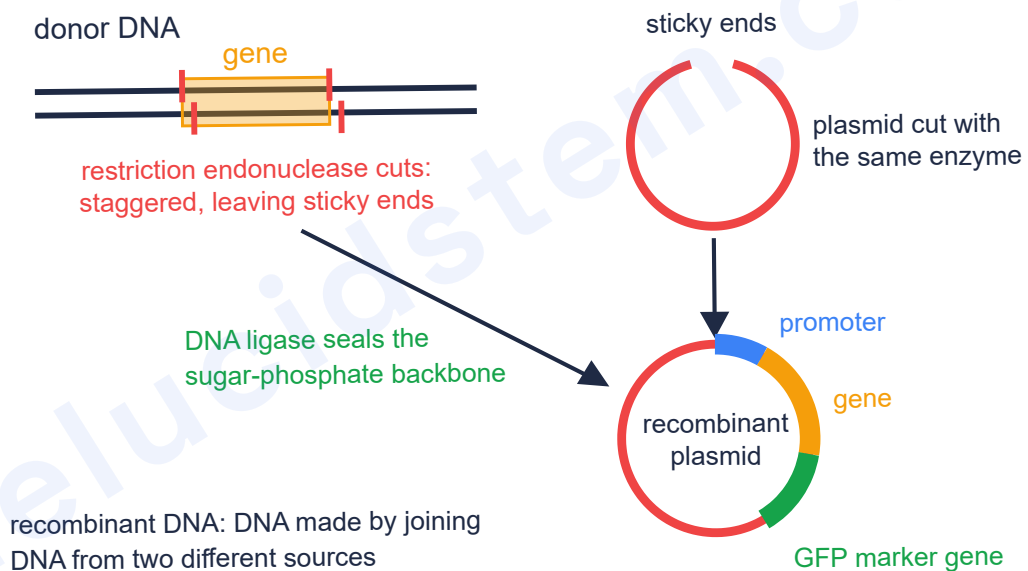


Fig. 1: a gene and a plasmid cut with the same restriction endonuclease are joined by DNA ligase to form a recombinant plasmid carrying a promoter and a fluorescent marker gene

Source, vector, switch, signal.

- ✓ **Source:** donor DNA (restriction enzyme), mRNA (reverse transcriptase + DNA polymerase) or chemical synthesis.
- ✓ **Vector:** plasmid cut with the **same** enzyme; **ligase** seals it.
- ✓ **Switch:** a **promoter** the host recognises, so the gene is transcribed.
- ✓ **Signal:** a **fluorescent marker** shows which cells express the DNA.

Watch out!

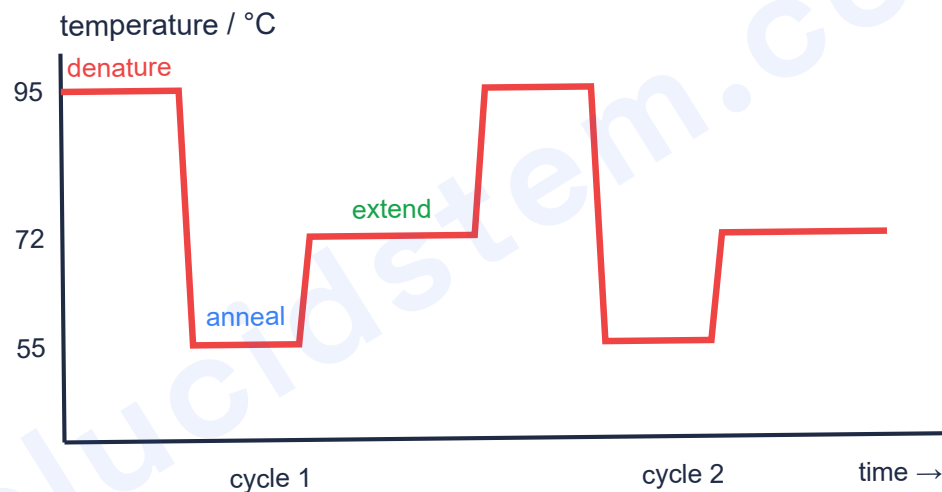
Reverse transcriptase makes DNA from an RNA template (the reverse of transcription). It does not make mRNA from DNA, and it is not the enzyme that cuts DNA.

Seeing the gene in the cell is not enough: the marker shows that the inserted DNA is being **expressed** (transcribed and translated), because the green colour only appears if a fluorescent protein has been made.

2 PCR and gel electrophoresis

Syllabus 19.1

The **polymerase chain reaction (PCR)** clones and amplifies a chosen section of DNA. A tube holds the template DNA, two **primers**, free DNA nucleotides, **Taq polymerase** and buffer, and a thermal cycler repeats three steps: **denaturation** at about 95 °C (hydrogen bonds break, strands separate), **annealing** at about 55 °C (primers bind to complementary sequences at each end of the target) and **extension** at about 72 °C (Taq polymerase adds nucleotides to the primers). Every cycle doubles the DNA, so n cycles give 2^n copies. Taq polymerase comes from a bacterium of hot springs and is **thermostable**, so it survives 95 °C. In **gel electrophoresis**, DNA is loaded into wells in an agarose gel; its phosphate groups make it **negatively charged**, so it moves towards the **anode**, and **shorter fragments move further** through the mesh of the gel.



copies double each cycle: $1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow \dots \rightarrow 2^n$ after n cycles

Taq polymerase is thermostable: it is not denatured at 95 °C

Fig. 1: two cycles of PCR in a thermal cycler; each cycle of denaturation, annealing and extension doubles the number of copies of the target DNA

Three temperatures, one direction.

- ✓ **95 °C** separate, **55 °C** primers bind, **72 °C** Taq extends.
- ✓ Copies = starting number $\times 2^n$ after n cycles.
- ✓ DNA is **negative**, so it runs to the **anode**.
- ✓ **Shorter** fragments travel **further**; compare with a ladder.

Watch out!

Primers are DNA, not RNA, in PCR, and there are two of them: one binds to each strand at opposite ends of the target, so the section between them is what gets copied.

Distance travelled is **not** proportional to length: it falls roughly with the logarithm of the fragment length, so always estimate sizes by comparing with a ladder on the same gel.

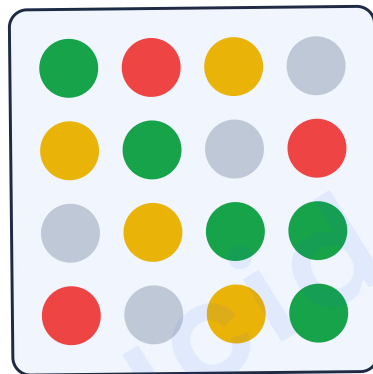
3 Microarrays and bioinformatics

Syllabus 19.1

A **microarray** is a small slide carrying thousands of spots, each holding many copies of a single-stranded DNA **probe** for one gene or sequence. To study **gene expression**, mRNA is extracted from two samples, converted to **cDNA** by reverse transcriptase, and labelled with different **fluorescent dyes**. The cDNA **hybridises** to complementary probes, unbound DNA is washed off, and a scanner records the colour and brightness of every spot: which genes are expressed in each sample, and how strongly. To **analyse genomes**, labelled fragments of genomic DNA are used instead, showing which genes or alleles two genomes share.

Bioinformatics databases store nucleotide sequences of genes and genomes, amino acid sequences of proteins and protein structures, so scientists anywhere can search, compare and use them.

microarray: each spot holds DNA probes for one gene



● green: sample 1 only

● red: sample 2 only

● yellow: both samples

● dark: neither sample

brighter = more mRNA for that gene

mRNA → cDNA (reverse transcriptase) → fluorescent label

→ hybridise to probes → wash → scan with a laser

sample 1 labelled green, sample 2 labelled red

Fig. 1: part of a microarray after hybridisation with cDNA from two samples; the colour of each spot shows in which sample its gene was being expressed

Which nucleic acid, which question.

- ✓ **mRNA** → **cDNA** on the array = which genes are being **expressed**.
- ✓ **Genomic DNA** on the array = which genes are **present**.
- ✓ Green / red = one sample only; **yellow** = both; dark = neither.
- ✓ Databases: **nucleotide** sequences, **amino acid** sequences, **protein structures**.

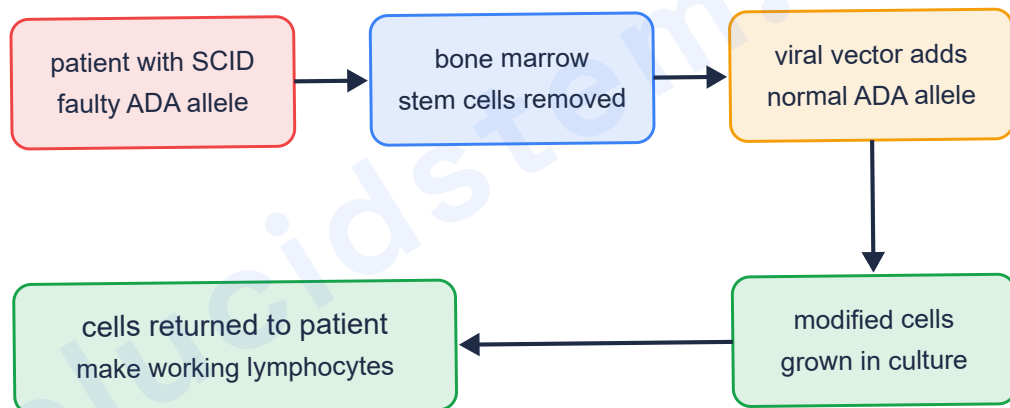
Watch out!

A dark spot in an expression study does not mean the gene is missing from the cells. It means the gene was not being **transcribed**, so there was no mRNA, and therefore no cDNA, to bind.

4 Genetic technology in medicine

Syllabus 19.2

Gene editing is a form of genetic engineering in which DNA is **inserted, deleted or replaced** at a **specific site** in the genome, for example using a guide RNA to lead a nuclease to the target. **Recombinant human proteins** such as **insulin** (diabetes), **factor VIII** (haemophilia) and **adenosine deaminase** (one form of SCID) are identical to the human proteins, made in large amounts and free from the infection risks of animal or donor sources. **Genetic screening** tests DNA for particular alleles, such as harmful **BRCA1 and BRCA2** alleles (breast cancer), the **Huntington's disease** allele and **cystic fibrosis** alleles, so people can make informed decisions and start treatment early. **Gene therapy** adds a working allele to a patient's cells using a vector, as in treatments for **SCID** and **inherited eye diseases**. All of these raise **social and ethical** questions about consent, privacy, discrimination, cost, safety and the limits of what should be changed.



somatic gene therapy: body cells only, not inherited

germ-line changes (gametes, embryos) would be inherited

Fig. 1: somatic gene therapy for SCID caused by lack of adenosine deaminase; the patient's own stem cells are given a normal allele outside the body and then returned

Protein, test, therapy, ethics.

- ✓ **Recombinant proteins:** insulin, factor VIII, ADA: identical to human, safe, plentiful.
- ✓ **Screening:** BRCA1/2, Huntington's, cystic fibrosis: informed choices, early action.
- ✓ **Gene therapy:** SCID (stem cells + viral vector), eye (AAV under the retina); somatic only.
- ✓ **Ethics:** consent, privacy, discrimination, cost, safety, embryos, germ line.

Watch out!

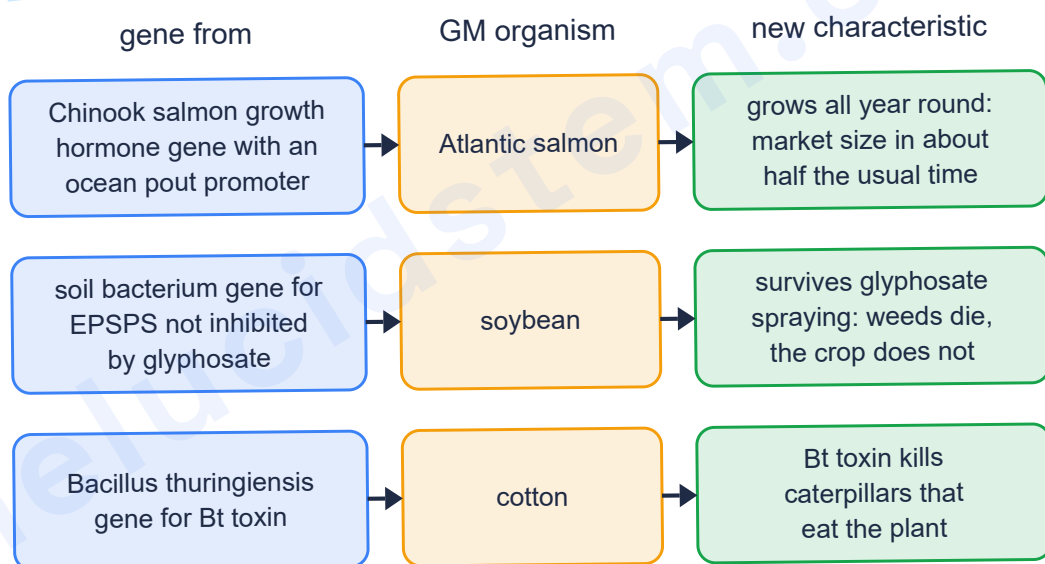
Bacteria cannot add sugar groups or modify proteins the way human cells do, so some recombinant human proteins, such as factor VIII, are made in cultured mammalian cells instead.

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5 GM crops and animals

Syllabus 19.3

Genetic engineering can help meet the **global demand for food** by improving the **quality and productivity** of farmed animals and crops. **GM salmon** carry a growth hormone gene from Chinook salmon with a promoter from ocean pout that keeps it switched on all year, so they reach market size in about half the time on less feed. **Herbicide-resistant soybean** carries a bacterial gene for a form of the enzyme EPSPS that is not inhibited by **glyphosate**, so fields can be sprayed to kill weeds without harming the crop. **Insect-resistant (Bt) cotton** carries a gene from *Bacillus thuringiensis* for a **Bt toxin** that kills caterpillars that eat it, so less insecticide is needed. The **ethical and social implications** include benefits to yield and farmers' incomes, and concerns about gene flow, effects on other species, resistance in weeds and pests, the cost of seed and control by large companies, animal welfare, labelling and consumer choice.



each transfer aims to raise the quality or productivity of food

Fig. 1: the three syllabus examples of genetically modified organisms in food production: where each gene comes from and what it changes

Gene, trait, benefit, risk.

- ✓ **GM salmon:** Chinook growth hormone gene + pout promoter: grows all year.
- ✓ **Soybean:** glyphosate-resistant EPSPS: spray weeds, not the crop.
- ✓ **Bt cotton:** Bt toxin kills caterpillars: less insecticide.
- ✓ Always weigh **benefits** against **gene flow, resistance, cost and choice**.

Watch out!

Herbicide-resistant crops do not kill weeds themselves. They survive the herbicide, which allows the farmer to spray it onto the growing crop. Bt crops are the ones that make their own insecticide.

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