

Topic	Genetic modification	Level	A Level
Outcomes	1. To correctly sequence the stages involved in the genetic modification of a bacterium		

Put these steps into the correct order by writing the letters (A-H) in the table provided. Once you have worked out the correct sequence draw a diagram (in the table provided) to illustrate what is happening in each step.

- A. These transformed cells are then spread out onto a Petri dish containing agar with ampicillin.
- B. The enzyme DNA ligase then joins the donor gene and the plasmid DNA permanently by forming covalent phosphodiester bonds which link up the sugar-phosphate backbone. The donor gene is now incorporated into the plasmid.
- C. The next stage is to insert the isolated gene into a vector. Bacterial plasmids, containing an ampicillin resistance gene, are isolated and cut open with the same restriction enzyme used to cut the donor DNA (in this example EcoR1)
- D. The cell membrane is disrupted and the nucleus is broken down in order to isolate the donor DNA from the rest of the cell's contents.
- E. The bacteria cells are then exposed to calcium ions (calcium chloride) which make the bacteria cell membranes more permeable, allowing uptake of the recombinant plasmids from the surroundings.
- F. Only the bacterial cells that have taken up the plasmid will be able to grow on the antibiotic media. These bacteria then divide by binary fission and with each division the plasmid and gene of interest are also replicated. If the gene product is required the transformed bacteria are cultured in an industrial fermenter.

- G. Plasmid DNA and the donor gene are mixed together and the unpaired bases of the foreign DNA and plasmid DNA join up by hydrogen bonding.
- H. Once the DNA has been isolated the required gene is cut out using the **restriction endonuclease enzyme** EcoR1, which cuts at recognition sites leaving two complementary 'sticky ends'.

TEACHER'S ANSWERS

- D. The cell membrane is disrupted and the nucleus is broken down in order to isolate the DNA from the rest of the cell's contents.
- H. Once the DNA has been isolated the required gene is cut out using the **restriction endonuclease enzyme** EcoR1, which cuts at recognition sites leaving two complementary 'sticky ends'.
- C. The next stage is to insert the isolated gene into a vector. Bacterial plasmids, containing an ampicillin resistance gene, are isolated and cut open with the same restriction enzyme used to cut the donor DNA (in this example EcoR1)
- G. Plasmid DNA and the donor gene are mixed together and the unpaired bases of the foreign DNA and plasmid DNA join up by hydrogen bonding.
- B. The enzyme DNA ligase then joins the donor gene and the plasmid DNA permanently by forming covalent phosphodiester bonds which link up the sugar-phosphate backbone. The donor gene is now incorporated into the plasmid.
- E. The bacteria cells are then exposed to calcium ions (calcium chloride) which make the bacteria cell membranes more permeable, allowing uptake of the recombinant plasmids from the surroundings.
- A. These transformed cells are then spread out onto a Petri dish containing agar with ampicillin.

F. Only the bacterial cells that have taken up the plasmid will be able to grow on the antibiotic media. These bacteria then divide by binary fission and with each division the plasmid and gene of interest are also replicated. If the gene product is required the transformed bacteria are cultured in an industrial fermenter.

An Outline of the Various Stages Involved in the Manipulation of Genetic Material

Step	Letter	Diagrammatic representation of step
1		
2		
3		
4		

Step	Letter	Diagrammatic representation of step
5		
6		
7		
8		

Progress: further resources on genes and inheritance are available here:
<http://thescienceteacher.co.uk/genes-and-inheritance/>