CAIE Biology A-level

Topic 19: Genetic Technology

Flashcards

State what is meant by ‘recombinant DNA’.

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DNA which has been produced by combining DNA from two or more different organisms.

Explain what is meant by ‘genetic engineering’.

Explain what is meant by genetic engineering.

Genetic engineering is the manipulation of genetic material. This may involve inserting, deleting or substituting DNA at specific parts of the genome. It is usually done to modify a characteristic in the host organism.

Outline the main stages of genetic engineering.

Outline the main stages of genetic engineering.

- Extracting genes from the DNA of an organism, or synthesising the required genes
- Placing these genes in an organism, either from the same species or a different one
- Checking that the host organism has taken up the gene
- Production of the gene product by the host organism

Name the three ways genes can be generated for genetic engineering.

Name the three ways genes can be generated for genetic engineering.

- Extracted directly from an organism's DNA using restriction endonucleases
- Generated from an mRNA sequence using reverse transcriptase
- Synthesising the gene artificially using nucleotides

What is the function of the polymerase chain reaction (PCR)?

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PCR is an *in vitro* method of cloning and amplifying a fragment of DNA.

Outline the substances required for PCR.

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- DNA fragment to be amplified
- Primers (short nucleotide sequences)
- DNA *Taq* polymerase
- Nucleotides

Describe how PCR works.

Describe how PCR works.

- The DNA fragment is separated into two strands. This is done by heating it to 95°C, which breaks the hydrogen bonds between bases.
- The machine then cools to 55°C. At this temperature, the primers can pair with their complementary bases, which are at the ends of the fragment. The primers form the start of a new DNA strand.
- The temperature is then increased to 72°C. *Taq* polymerase synthesises the rest of the new DNA strands, joining the nucleotides which have paired with their complementary base.
- This cycle repeats, doubling the amount of DNA each time.

Why is *Taq* polymerase used?

Why is *Taq* polymerase used?

Taq polymerase is resistant to heat (because it comes from bacteria which live in hot springs). Therefore, it does not denature at high temperatures.

Explain why primers are needed for PCR.

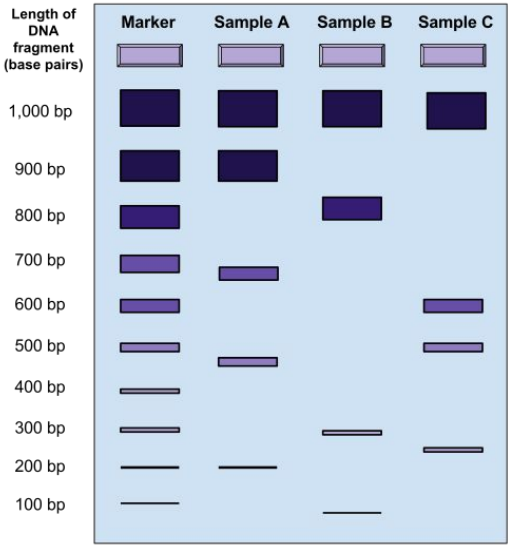
Explain why primers are needed for PCR.

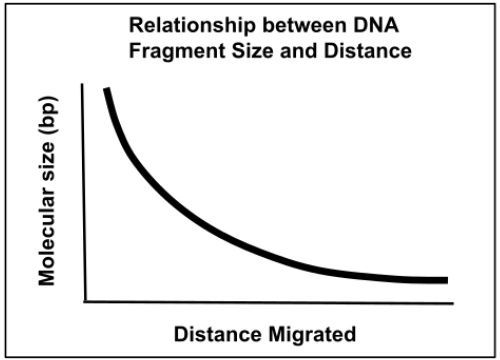
The primers provide a starting point for *Taq* polymerase. There are two types of primer, each one complementary to the 3' end of the DNA strands. The primers also prevent the two DNA strands joining back together after they have been separated.

What is the function of gel electrophoresis?

What is the function of gel electrophoresis?

To separate DNA fragments, nucleic acids and proteins according to their size and charge.





Outline the process of gel electrophoresis of DNA to identify a particular allele.

Outline the process of gel electrophoresis of DNA to identify a particular allele.

- The DNA is extracted and may be amplified using PCR
- Restriction endonucleases are used to cut the DNA into fragments
- The DNA fragments are separated by an electrical voltage passed across the gel. Smaller fragments of DNA move faster and further than larger fragments
- The DNA strands are then separated by heat or an alkali
- Probes are added to bind to specific DNA sequences (alleles). The probes are usually radioactive, so they show up on an X-ray film
- The alleles of interest are identified

Outline how different alleles can be distinguished by gel electrophoresis of proteins.

Outline how different alleles can be distinguished by gel electrophoresis of proteins.

- Different alleles code for slightly different amino acid sequences
- The charge of the proteins will differ depending on the R groups of the amino acids present
- The proteins can be separated according to charge

What is a DNA probe?

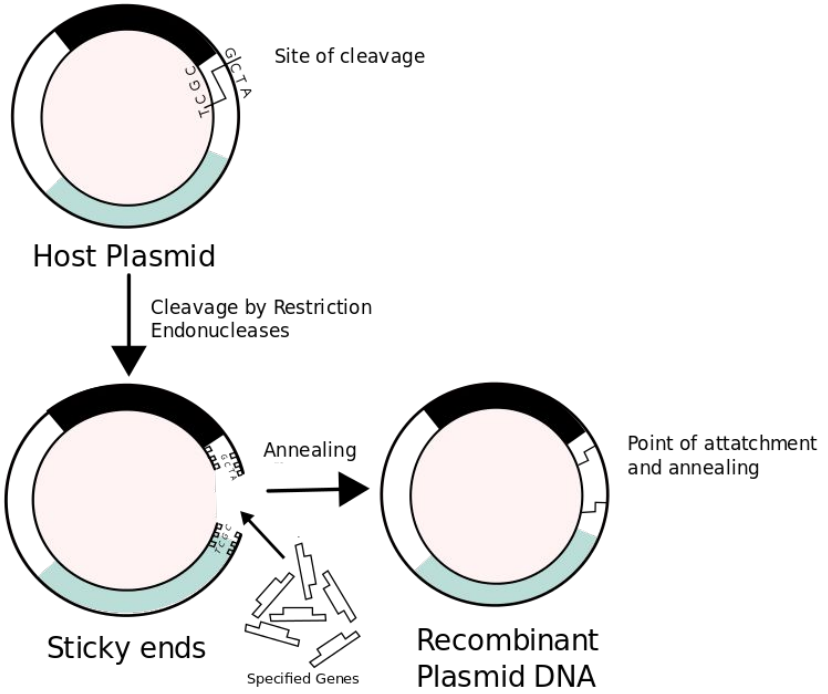
What is a DNA probe?

- A short, single-stranded sequence of nucleotides with a label attached. It is complementary to the base sequence of a particular gene in the DNA.
- The label may be fluorescent or radioactive. The label is only visible/detectable when the probe has bound to the DNA sequence.

What is a plasmid?

What is a plasmid?

A plasmids is a circular DNA structure found in bacteria. It is a **vector** used to carry DNA into the host cell.



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Why are plasmids used in genetic engineering?

Why are plasmids used in genetic engineering?

- They are easy to isolate and insert DNA into
- Plasmids often have a gene for antibiotic resistance, which can be used to identify bacteria that have taken up the plasmid
- The plasmid DNA is double-stranded, so can carry prokaryotic and eukaryotic DNA

Why do promoters need to be transferred along with the desired gene?

Why do promoters need to be transferred along with the desired gene?

Promoters control the expression of the gene. Transcription factors and RNA polymerase bind to the promoter sequence so transcription can occur. Without this, the gene cannot be transcribed.

Describe how marker genes are used in gene technology.

Describe how marker genes are used in gene technology.

Marker genes produce a gene product or effect which is easily identifiable. They are present on the plasmid, along with the transferred gene. Therefore, they can confirm that the host cell has taken up the plasmid. For example, fluorescent markers may be used, or the gene may produce a substance that can stain.

State the role of restriction endonucleases.

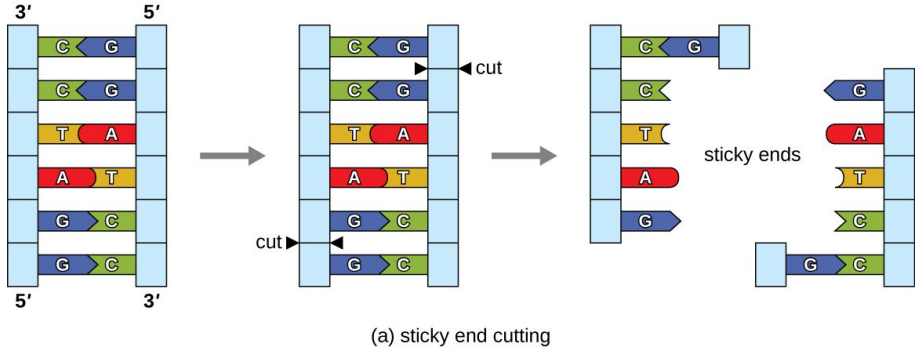
State the role of restriction endonucleases.

Restriction endonucleases are enzymes which cut viral DNA at specific sites. They are part of a bacterium's defence against pathogens, but can also be used for gene technology.

Describe the types of cut produced by restriction endonucleases.

Describe the types of cut produced by restriction endonucleases.

- Straight cut across the double stranded DNA - this produces **blunt ends**
- Staggered cut across the DNA - this produces **'sticky' ends**, which means there is a short sequence of unpaired nucleotides on each piece of DNA.



Explain how reverse transcriptase is used.

Explain how reverse transcriptase is used.

Reverse transcriptase is an enzyme found in some viruses. It converts mRNA into single-stranded DNA, which can then be used to produce double-stranded complementary DNA (cDNA). It essentially reverses the process of transcription.

Describe how DNA ligase is used in genetic engineering.

Describe how DNA ligase is used in genetic engineering.

DNA ligase joins the desired DNA fragment to the opened-up plasmid. As they have both been cut by the same restriction endonuclease, they have complementary sticky ends. DNA ligase forms the phosphodiester bonds between adjacent nucleotides to join the DNA.

Outline how microarrays are used to analyse genomes.

Outline how microarrays are used to analyse genomes.

- The DNA from each sample is denatured (separating the strands), and then cut into fragments using restriction endonucleases
- Each fragment is labelled with a fluorescent tag
- The DNA is loaded onto the microarray. The microarray contains thousands of probes, which hybridise with their complementary genes
- UV light is used to see where the DNA has hybridised to the probe. The alleles present in the genomes can be identified and compared

How can microarrays be used to assess gene expression?

How microarrays be used to assess gene expression?

Messenger RNA is extracted from the cell and is converted back into cDNA using reverse transcriptase and DNA polymerase. This DNA is tagged and hybridised with probes. The fluorescence at each spot shows how much mRNA there was and therefore the level of expression of the gene.

State what is meant by the term ‘bioinformatics’.

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The collection, storage and analysis of genomes from thousands of organisms.

How is bioinformatics used following genome sequencing?

How is bioinformatics used following genome sequencing?

Once the genomes have been sequenced, large databases are created with the biological information. This allows for comparisons and analyses to determine similarities and differences between genomes.

Give some examples of human proteins that have been produced by recombinant DNA technology.

Give some examples of human proteins that have been produced by recombinant DNA technology.

Insulin, factor VIII and adenosine deaminase. These proteins can be used to treat diabetes, haemophilia and severe combined immunodeficiency (SCID).

Outline the benefits of producing human proteins by recombinant DNA technology.

Outline the benefits of producing human proteins by recombinant DNA technology.

- The human version of the protein can be produced, rather than that from an animal (e.g. pig)
- The rate at which the proteins can be produced is much faster than other methods e.g. blood transplants
- The supply is reliable
- The gene product can be easily extracted and purified

Give examples of genetic conditions that can be screened for.

Give examples of genetic conditions that can be screened for.

- Sickle-cell anaemia
- Breast cancer (*BRCA1* and *BRCA2*)
- Haemophilia
- Cystic fibrosis
- Huntington's disease

Why is genetic screening carried out?

Why is genetic screening carried out?

- It can be used to test for and diagnose genetic conditions, for example in an unborn fetus or an individual with a family history of a genetic condition

- It may be used in personalised medicine to determine the best treatment for a particular genotype

What is gene therapy?

What is gene therapy?

Treatment of a genetic condition by altering the individual's DNA.

Give some examples of vectors that may be used in gene therapy.

Give some examples of vectors that may be used in gene therapy.

- Viruses
- Liposomes
- Naked DNA

Outline the challenges faced when choosing an appropriate vector for gene therapy.

Outline the challenges faced when choosing an appropriate vector for gene therapy.

- Retroviruses insert their DNA randomly into the host cell. This can disrupt other genes, potentially leading to inactivation of tumour suppressor genes or activation of oncogenes
- Viral vectors are limited in how much can be carried
- Antiviral immunity can prevent viral vectors delivering the DNA to the target cells
- Naked DNA may not be efficiently delivered into cells or incorporated into the host cell genome

What are the social and ethical considerations of using gene therapy and genetic testing?

What are the social and ethical considerations of using gene therapy and genetic testing?

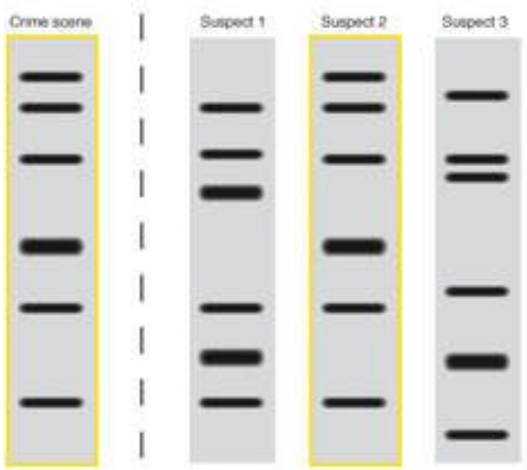
- **Gene therapy**
  - Moral acceptability of gene therapy and how it may be exploited (e.g. ‘designer babies’)
  - Often expensive so it may only be available to the rich

- **Genetic testing**
  - Psychological burdens on the individual and their families
  - When screening unborn fetuses, the method of obtaining fetal DNA can cause miscarriage
  - If the test for a genetic condition is positive or if the fetus is not the desired sex, the parents may choose to abort
  - It can affect insurance covers (e.g health and life)

How can PCR and DNA testing be used in forensic science?

How can PCR and DNA testing be used in forensic science?

DNA found at crime scenes can be amplified using PCR and tested to see if it matches the DNA of a suspect.



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https://www.mrgscience.com/topic-35-genetic-engineering-and-biotechnology.html

Why is genetic engineering used in crops and livestock?

Why is genetic engineering used in crops and livestock?

By manipulating the genome of plants and livestock, the quality and the yield can be increased. This is important for meeting the world's increasing food demands.

Describe how Bt maize is different from regular maize.

Describe how Bt maize is different from regular maize.

Bt maize is resistant to certain insects (e.g. caterpillars) which improves its yield. It expresses the Bt toxin, which comes from a species of bacteria. The Bt toxin is selective to certain pests and does not harm other organisms.

Explain how rice may be enhanced with vitamin A.

Explain why rice may be enhanced with vitamin A.

Golden rice is a GMO that synthesises high amounts of β-carotene, which can be converted to vitamin A.

Normal white rice does not contain vitamin A, so the rice is transformed using β-carotene genes from daffodils and bacteria. These genes and a promoter are inserted into a plasmid and introduced into *Agrobacterium* bacteria. The bacteria are mixed with rice embryos and the embryos that take up the bacterial DNA with the genes for β-carotene are selected.

This type of rice can be grown areas where there is a deficiency of dietary vitamin A.

Describe how salmon have been genetically modified.

Describe how salmon have been genetically modified.

The gene for regulating growth hormone from the Pacific Chinook salmon, and a promoter from ocean pout are inserted a fertilised egg from Atlantic salmon. The growth hormone is expressed constantly causing the salmon grow faster.

How can genetic engineering increase the production of crops?

How can genetic engineering increase the production of crops?

- Crops may be genetically engineered to contain genes for herbicide resistance; they won't be harmed when herbicides are sprayed to kill the weeds. This is seen in tobacco and oil seed rape plants
- Some crops may be engineered to be resistant to insecticides, like Bt maize and cotton.

Outline the social and ethical implications of using GMOs in food production.

Outline the social and ethical implications of using GMOs in food production.

- GMOs may trigger allergies
- There are moral issues associated with the manipulation of genes
- Changes to farming practices - farmers now buy the GMO seeds from the patent owner
- Increased use of herbicides and insecticides can damage many insect species, including beneficial ones. It may also lead to resistance in weeds and pests, damage the soil and affect human health