Edexcel International A Level (IAL) Biology

Your notes

Gene Technology

Contents

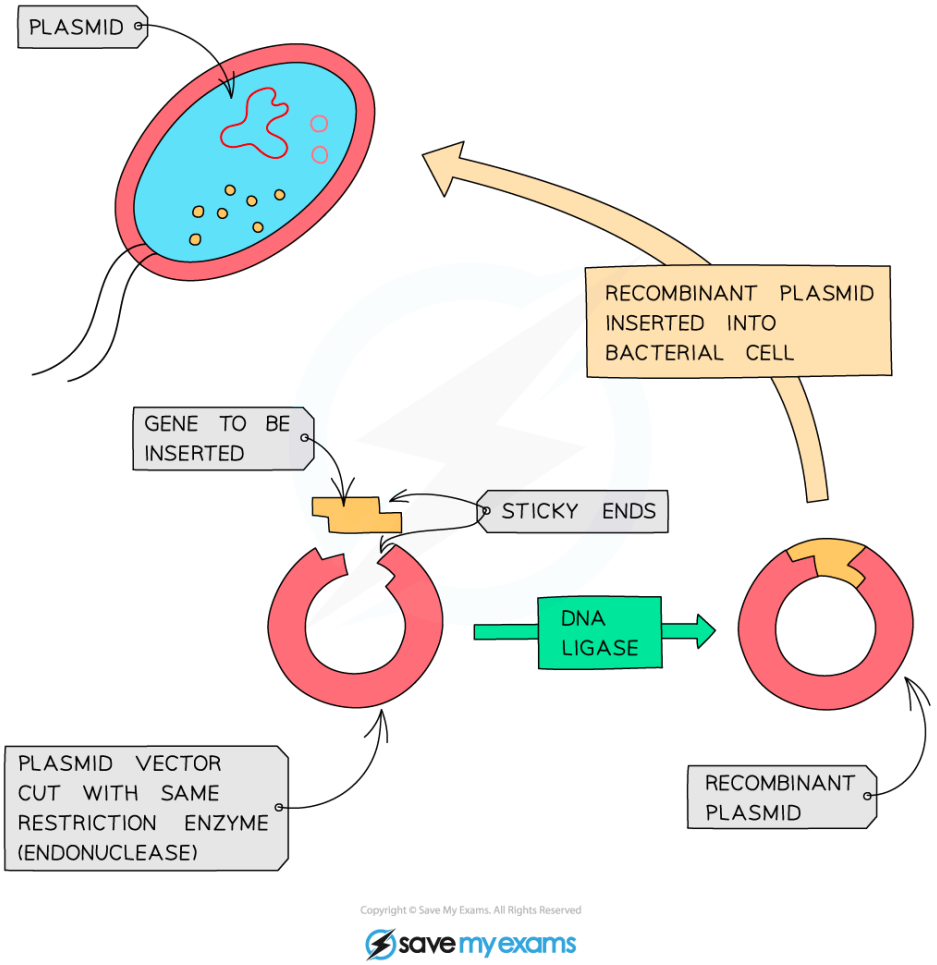
Your notes

Drug Production from Genetically Modified Organisms

- Genetic engineering is a technique used to deliberately modify a specific characteristic of an organism
  - The technique involves **removing a gene** that codes for a desired characteristic from **one organism** and **transferring** the gene **into another** organism where the **desired gene is then** expressed
- The genetically engineered organism is said to contain **recombinant DNA** and will be a **genetically modified organism** (GMO)
- Micro-organisms, plants and animals have been genetically engineered to produce proteins used in medicine

Genetically modified micro-organisms

- Restriction enzymes are used to **remove** the gene coding for a desired protein from an organism's genome
  - The protein coded for here will be responsible for the **characteristic desired** in the GMO e.g. the ability to produce insulin
- Many **copies** of the gene are made using the **polymerase chain reaction**, or **PCR**
  - The enzyme DNA polymerase is used to join free nucleotides into new strands of DNA that are complementary to the original strand
- These copies are **inserted into** small loops of DNA called **plasmids,** which then **transfer the copies** into micro-organisms
  - The plasmids are said to be DNA **vectors**
  - The enzyme DNA ligase catalyses the joining of the desired gene to the plasmid vector
- The genetically modified micro-organisms are **grown in large fermenters** containing nutrients, enabling them to **multiply** and produce **large quantities** of the new protein
- The protein can be **isolated** and **purified** before being packaged and distributed
  - **Human insulin** and **human blood clotting factors** are examples of medicinal proteins produced by genetically modified bacteria



The human insulin gene can be inserted into bacterial plasmid vectors which are then transferred into bacterial cells

Genetically modified plants

- A similar process can be used to insert desired genes from other organisms into plant cells
- After the gene is inserted into a plasmid and then transferred to a bacterial cell, the bacteria can be used to **infect plant cells;** the bacterium acts as a **vector** for **introducing the gene into the plant DNA**
  - Note that this isn't the only method of introducing new genes into plant cells
    - Another method involves a 'gene gun'; tiny pellets are coated with the desired DNA and then fired into the plant cells
- The gene is transferred from the bacterial cell **into the plant cell nucleus**, after which the plant cell is stimulated to multiply and grow into an **adult plant**
  - **Each cell** of the plant **contains a copy** of the gene coding for the desired protein

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- The protein can now be **purified** from the plant tissues, or the plant can be **eaten** to deliver the drug
    - **Human insulin** and a **cholera vaccine** are examples of drugs produced by modified plants

Genetically modified animals

- The gene that codes for the desired protein is injected into the **nucleus of a** zygote
- The zygote is **implanted** into the uterus of a surrogate animal where it develops into an **adult animal**
    - **Every cell** of this genetically modified animal will **contain a copy** of the gene coding for the desired protein
- The protein can be **purified** from **e.g. the milk** of the animal
    - **Human blood clotting proteins** can be produced from the milk of genetically modified animals

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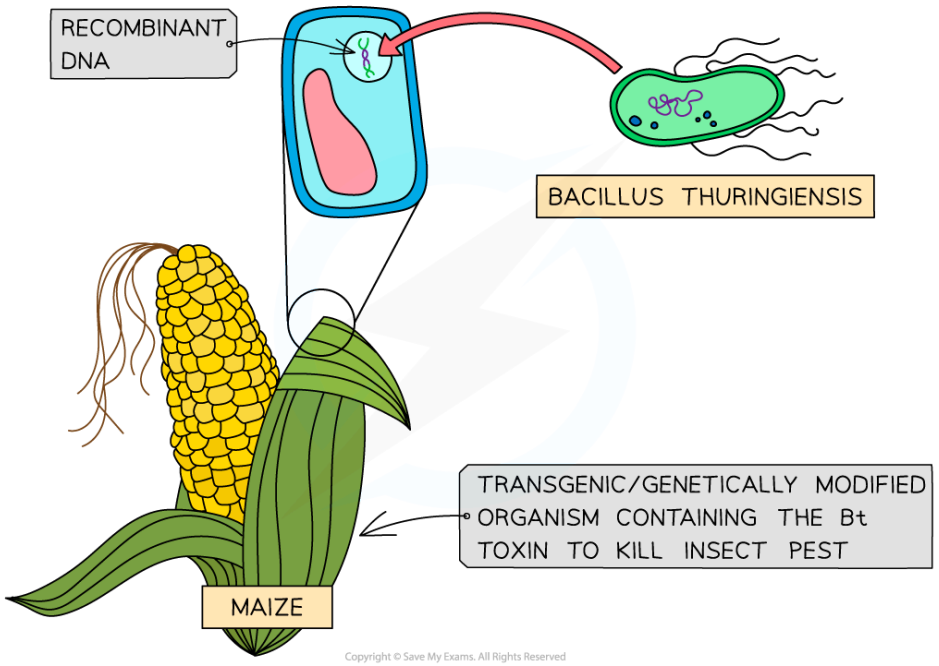
Recombinant DNA

- The genetic code is the basis for storing instructions that, alongside environmental influences, **dictate the characteristics of organisms**
- The genetic code is **universal**, meaning that almost every organism uses the same four nitrogenous bases A, T, C, and G
  - The universal nature of the genetic code means that the **same** codons **code for the same amino acids in all living things;** genetic information is therefore transferable between species
  - The mechanisms of transcription and translation are also **universal** which means that the transferred DNA can be translated within cells of another species
- Scientists can artificially change an organism's DNA by combining lengths of nucleotides from **different sources;** typically the nucleotides are from different species
- The altered DNA, with the introduced nucleotides, is called **recombinant DNA**
- If an organism contains nucleotide sequences from a different species it is known as a **transgenic** organism or a **genetically modified organism** (GMO)



Transferring genes from bacteria into the DNA of maize plants creates recombinant DNA

- Producing a transgenic organism involves the following process
  - **Identification** of the **desired gene**
    - This gene will code for a desired characteristic, e.g.

- Pest resistance genes in crops
- The human insulin gene

- **Isolation** of the desired gene by, e.g.
  - Using an enzyme called **reverse transcriptase** to convert a desired length of mRNA back into DNA; DNA produced in this way is known as complementary DNA, or **cDNA**
  - Cutting the gene from its location on a chromosome using enzymes called **restriction endonucleases**
  - Designing and building synthetic DNA sequences in a lab
- **Multiplication** of the gene, i.e. producing many copies, or clones; this can be carried out using the polymerase chain reaction (PCR)
  - PCR machines known as thermocyclers use free nucleotides, DNA polymerase, and DNA primers to produce many identical copies of a desired gene
- **Transfer** of the desired gene into another organism's DNA using a **vector,** e.g. DNA plasmids, viruses, or fatty envelopes known as liposomes
  - Once another organism has taken up the vector it is said to be **transformed**
- **Identification** of the cells that contain the new gene by using a **marker gene** alongside the desired gene; this means that any cells that take up the desired gene will take up the marker gene as well e.g.
  - Antibiotic resistance; transformed cells will survive if treated with a specific antibiotic
  - Fluorescence; transformed bacterial cells will fluoresce under UV light

- Once the transformed cells have been identified they can be **cloned**, ensuring that all new cells contain copies of the desired gene
  - In the case of bacteria this can be carried out in a large container known as a fermenter

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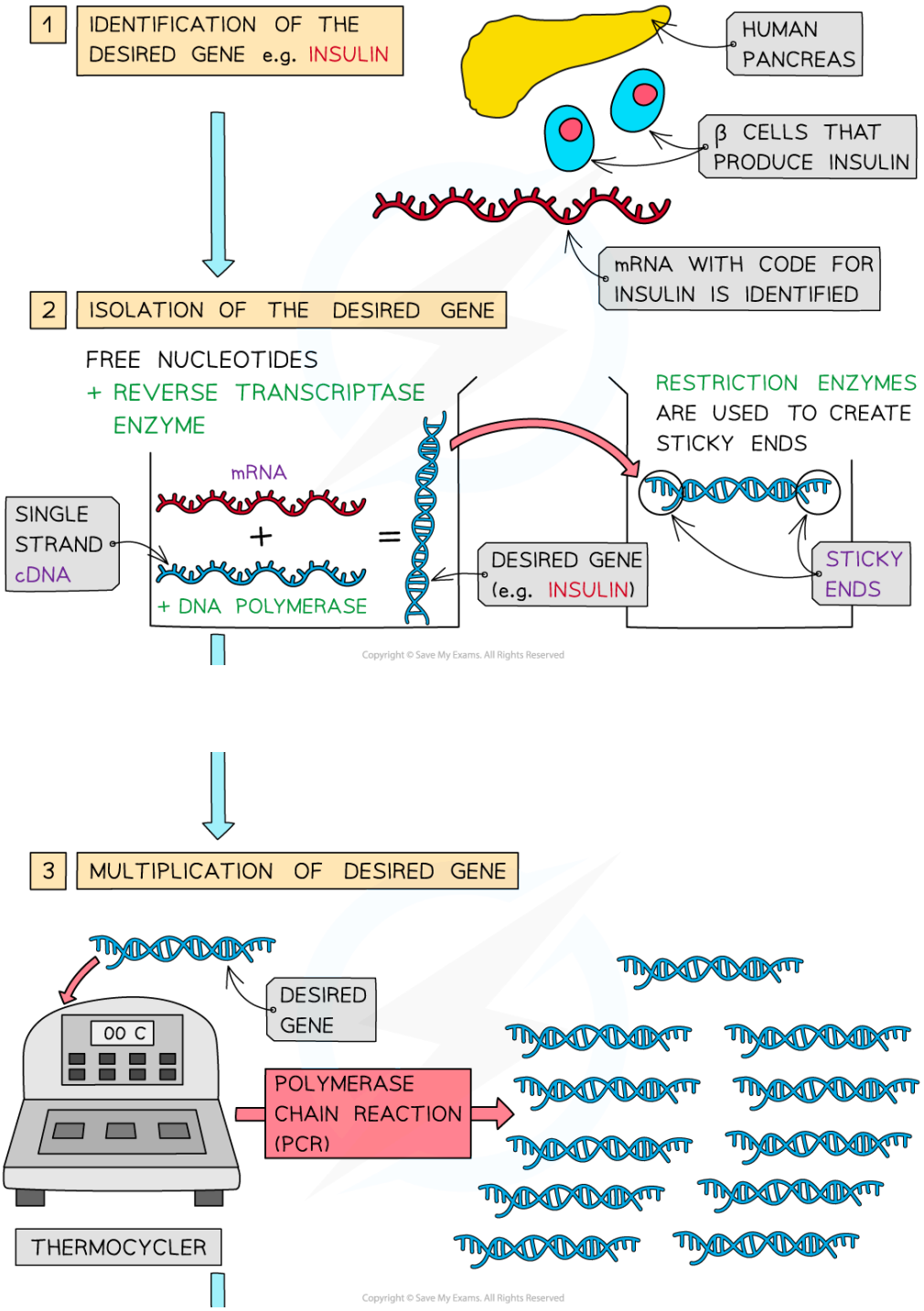
1
IDENTIFICATION OF THE DESIRED GENE e.g. INSULIN
HUMAN PANCREAS
β CELLS THAT PRODUCE INSULIN
mRNA WITH CODE FOR INSULIN IS IDENTIFIED
2
ISOLATION OF THE DESIRED GENE
FREE NUCLEOTIDES
+ REVERSE TRANSCRIPTASE ENZYME
RESTRICTION ENZYMES ARE USED TO CREATE STICKY ENDS
mRNA
SINGLE STRAND cDNA
+
+ DNA POLYMERASE
=
DESIRED GENE (e.g. INSULIN)
STICKY ENDS
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3
MULTIPLICATION OF DESIRED GENE
DESIRED GENE
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POLYMERASE CHAIN REACTION (PCR)
THERMOCYCLER
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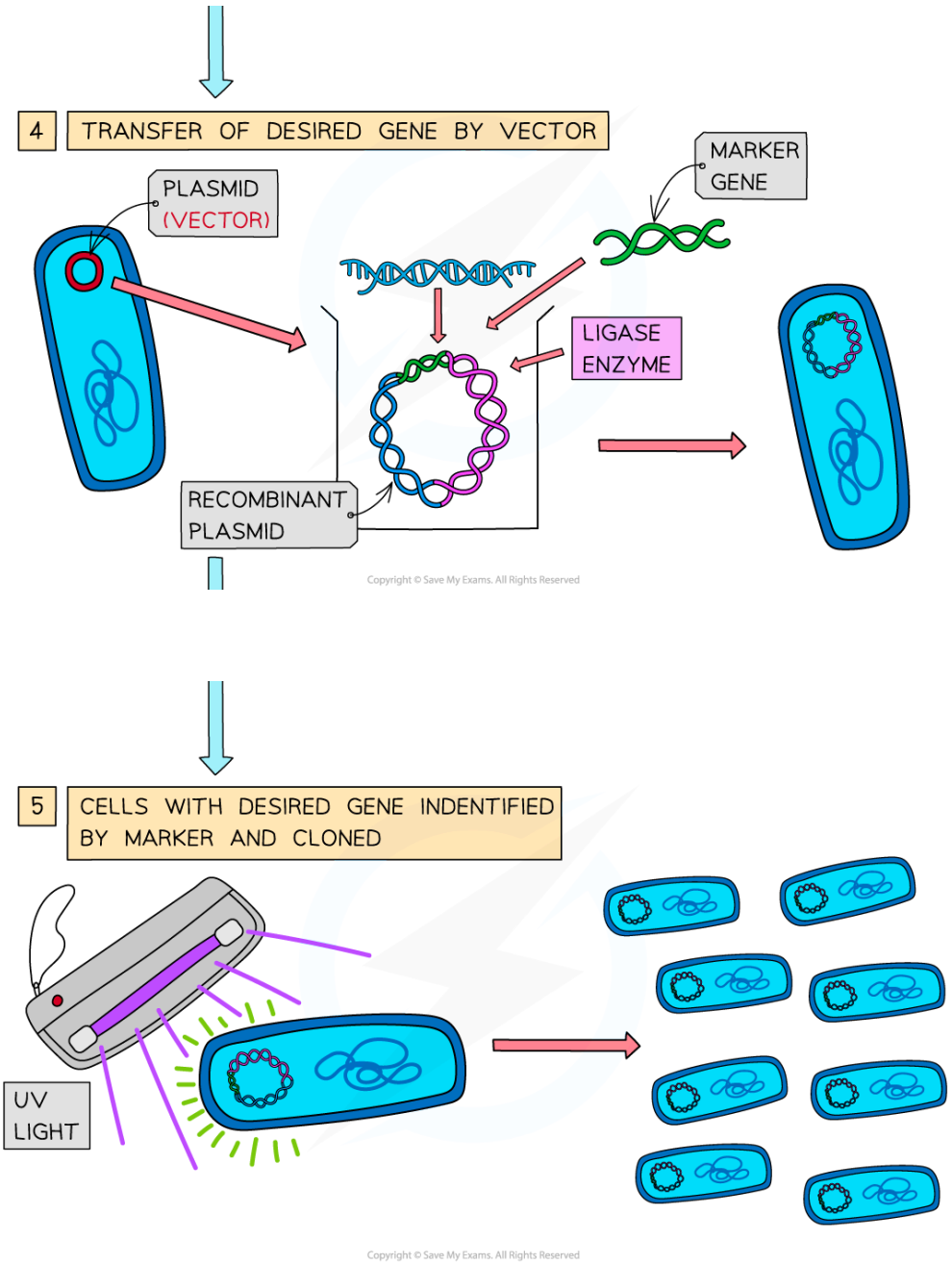

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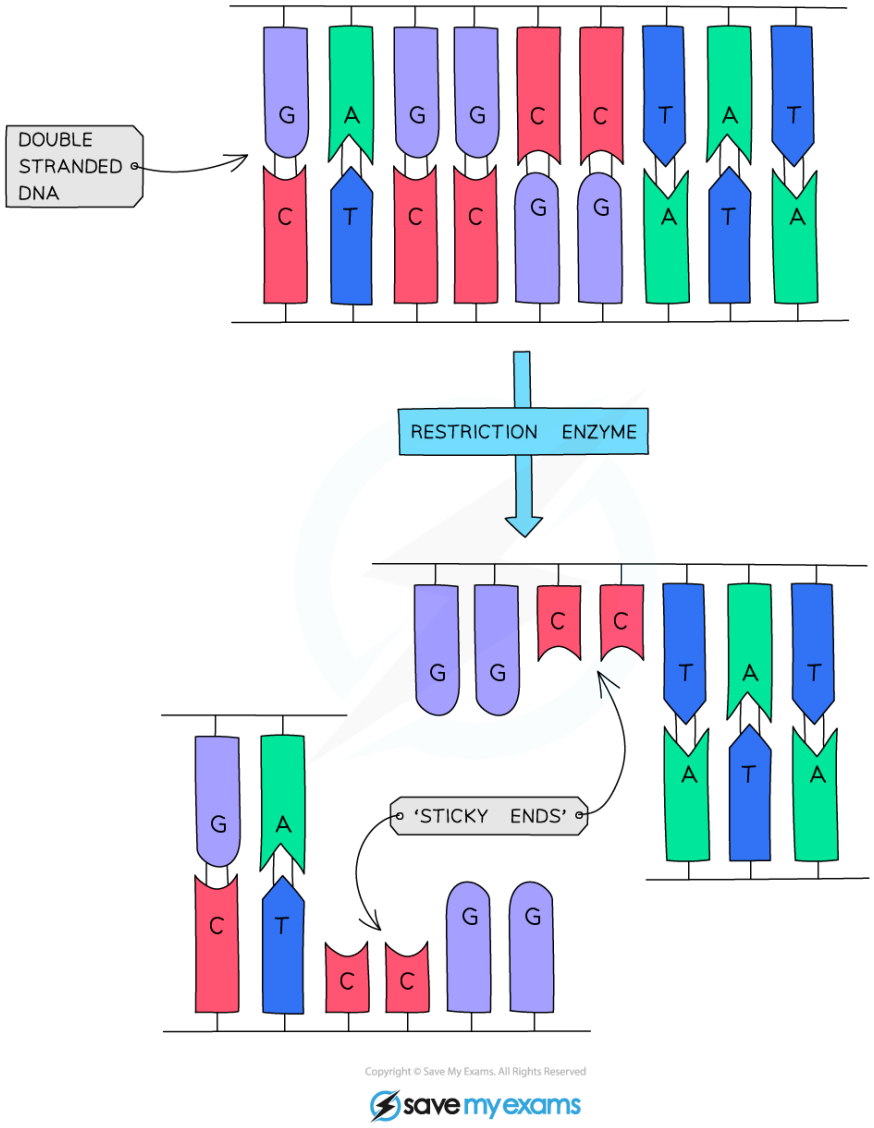
DNA can be transferred from one organism to another to produce recombinant DNA; this process involves identification, isolation, multiplication, and transfer of the desired gene, followed by identification and cloning of the transformed organisms

Isolating the desired gene using restriction endonucleases

- **Restriction endonucleases** are enzymes that cut DNA
  - They are sometimes referred to as **restriction enzymes**
- There are many different restriction endonucleases, each of which binds to a **specific** sequence of bases known as a **restriction site** on DNA, e.g. the restriction endonuclease HindIII will always bind to the base sequence AAGCTT

- Restriction endonucleases separate DNA at restriction sites by cutting the **sugar-phosphate backbone** in an uneven way; this leaves exposed single-stranded sequences of bases known as '**sticky ends**'
  - Sticky ends result in one strand of the DNA fragment being longer than the other strand
- The sticky ends make it easier to insert the desired gene into another organism's DNA or into **vector DNA** as they can easily form hydrogen bonds with complementary base sequences that have been cut with the **same restriction endonucleases**



Restriction enzymes produce a jagged cut at a restriction site, leaving 'sticky ends'

Inserting the desired gene into a vector using DNA ligase

- Once the desired gene has been cut from DNA using the relevant restriction endonuclease, it can then be transferred into the DNA of a **vector**, e.g.

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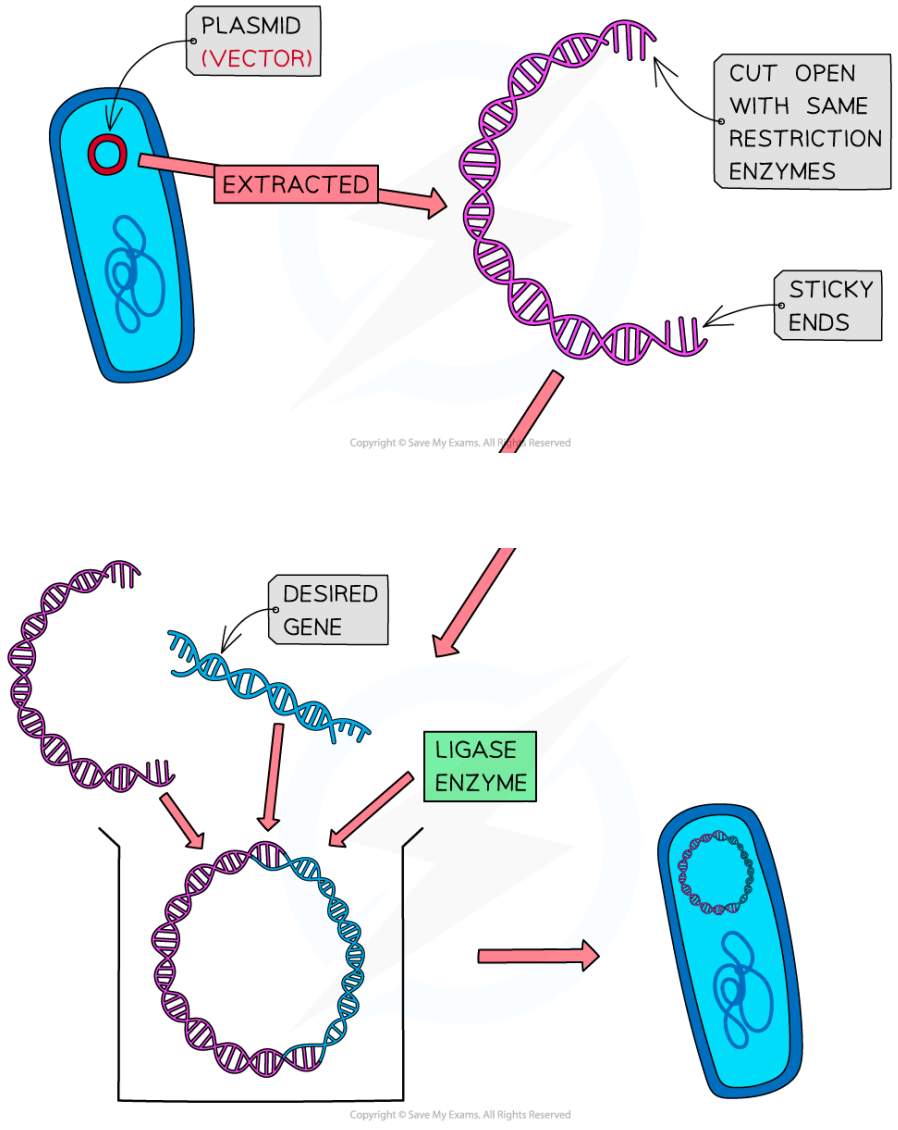
- A DNA plasmid
- A vector organism such as a virus or bacterium

- The DNA of the vector will be cut using the **same restriction endonuclease** as the desired gene, leaving **complementary sticky ends**
- The enzyme **DNA ligase** is used to catalyse the formation of **phosphodiester bonds** between the sugar-phosphate backbone of the desired gene and that of the vector DNA
  - If this is carried out using a plasmid, the plasmid will be known as a **recombinant plasmid**



DNA ligase is used to join the isolated gene to the vector DNA

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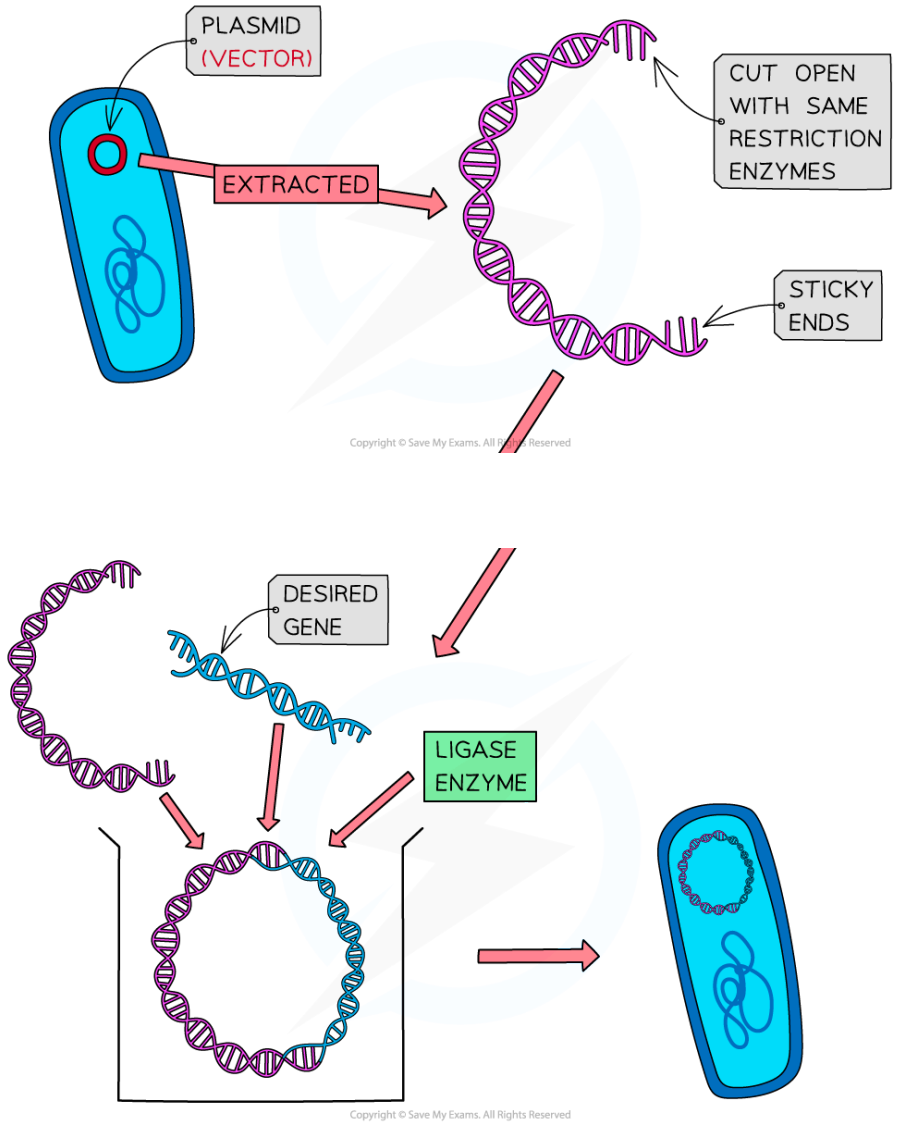
Transfer of Recombinant DNA into Other Cells

- Sections of DNA can be transferred from one organism's DNA to that of another, creating **recombinant DNA**
- In order to create recombinant DNA, desired genes, such as the gene for human insulin, need to be **transferred into new types of cell**; there are several different ways of doing this, e.g.
  - Vectors, e.g.
    - DNA plasmids
    - Viruses
    - Liposomes
  - Gene guns
  - Microinjection
- Once a section of DNA has been transferred into a new cell it needs to be **incorporated into the cell's genetic material** in order to be transcribed and translated; the success rate for transferred genes entering the nucleus of a eukaryotic cell is currently **very low**
  - The problem of transferred genes not reaching the nucleus is a barrier to the success of **gene therapy**
    - Gene therapy is a medical technique that involves inserting non-mutated genes into the cells of individuals with genetic disorders

Plasmid vectors

- Plasmids are small, circular rings of double-stranded DNA that can be found in some bacterial cells, as well as some other cell types
- To insert the desired gene into the circular DNA of a plasmid, it is cut open using a **restriction endonuclease**
- **DNA ligase** is then used to join the desired gene to the plasmid DNA; this creates a **recombinant plasmid**
- The **recombinant plasmid DNA** can then be **transferred into other cells**, usually bacteria, by a process called **transformation**
  - Only a small proportion of bacteria will become transformed and therefore marker genes such as antibiotic resistance are used to identify them
    - Scientists can **modify** or **design** bacterial plasmids so that they contain one or more **marker genes,** e.g. genes for antibiotic resistance
- Transformation can occur by

- Bathing the plasmids and bacteria in an ice-cold calcium chloride solution and then briefly incubating at 40°C; this makes the bacterial outer membrane permeable
- Electroporation; the bacteria are given a small electrical shock, making the membrane porous



Plasmid vectors can be used to transform bacterial cells

Viral vectors

- Viruses reproduce by **inserting their DNA into the cells of other organisms**, making them ideal vectors for the process of creating transformed cells
- Viruses exist that infect animal cells, plant cells, and bacterial cells, so viral vectors can be used to transform **many cell types**
- Viruses used as vectors need to be **non-harmful** to the cells being transformed

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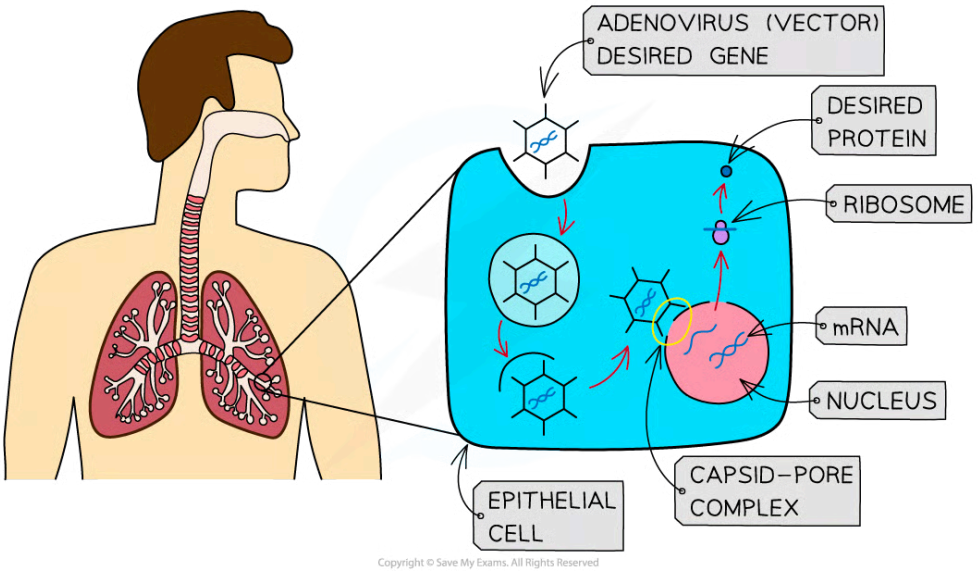
- Viruses are useful for ensuring that transferred DNA **reaches the nucleus** of cells, but despite being harmless they can sometimes **initiate an immune response**
- Viruses can be used as vectors in the process of **gene therapy**, which can be used to treat genetic diseases such as cystic fibrosis in humans



Viral vectors can be used to insert genes into human cells

Liposome vectors

- Liposomes are small, spherical vesicles, surrounded by a **phospholipid bilayer**
- Liposomes can be used as vectors because they can **fuse with the cell surface membranes** of host cells
- These vesicles can also be used in gene therapy to carry non-mutated genes into host cells

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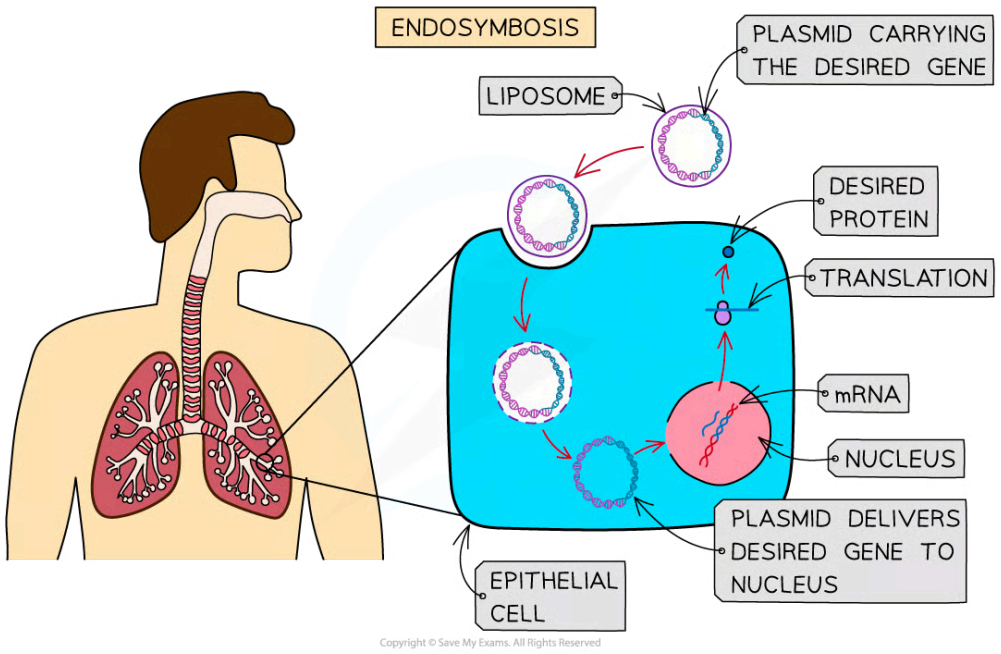

Liposomes can enter cells by fusing with the cell surface membrane. Note that you do not need to know the term endosymbiosis here

Gene guns

- The fragments of DNA containing the desired gene can be used to **coat tiny pellets** of gold or tungsten which are then **fired at high speed** into the cells that are to be transformed
- Cells that avoid damage are able to incorporate the new DNA into their genome

Microinjection

- A fine glass **micropipette** is used to transfer DNA into a cell
- This is a common laboratory technique for transferring genetic material into animal cells for the creation of transgenic animals

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Identification of Active Genes

- Microarrays are laboratory tools used to **identify active genes**
  - Active genes are genes that are **expressed**; **mRNA is transcribed** from them and the resulting mRNA strand is **translated into a polypeptide**
- Microarrays can be used to identify active genes in **thousands of gene samples** at a time
- Microarrays are used for
  - Medical diagnosis and treatment, e.g. identification of harmful mutations
  - Biotechnology, e.g. identifying genes for the process of producing recombinant DNA
  - Forensic analysis, e.g. in criminal investigations
- A microarray consists of a small piece of glass, plastic or silicon that has **DNA probes** attached to many spots, called **gene spots**, in a grid pattern
  - DNA probes are short, single stranded lengths of DNA linked to an **easily identifiable label** such as a fluorescence protein or a radioactive tag; these single stranded probes **bind to any complementary sequences** present in a DNA sample, indicating their presence by fluorescing or under an x-ray
  - There can be 10 000 or more spots per cm^2
- When producing a microarray, scientists compare experimental samples of genetic material with a **known reference sample**, e.g. a genetic sample taken from an individual known to have a particular disease mutation
- When a microarray is used to **analyse genomes**
  - Samples of genetic material, known as **chips**, are collected from
    - A **reference** source, e.g. an individual known to have a particular genetic mutation; this provides a control sample for comparison
    - An **unknown** source, e.g. a patient to be diagnosed
      - Note that many unknown samples can be simultaneously compared to a single reference sample
  - Collecting **mRNA** rather than DNA at this stage enables **active genes to be identified**; only active genes will be undergoing the process of **transcription into mRNA**
  - Enzymes called **reverse transcriptase enzymes** are used to convert the mRNA back into DNA; the DNA produced in this way is known as **complementary DNA**, or **cDNA**
  - The cDNA samples are labelled, e.g. using **fluorescence labels**

- The reference samples are labelled with a different label to the unknown experimental samples
    - Usually the **reference samples fluoresce green** while the **unknown samples fluoresce red**

- Once the reference and unknown samples are mixed together, they are then allowed to hybridise with the probes on the microarray
    - After a set period of time any DNA that did not hybridise with the probes is washed off
- The microarray is then examined using ultraviolet light which causes the tags to fluoresce, or scanned; **colours are detected** by the computer and the information is analysed
- The fluorescence colour detected indicates where **hybridisation** has occurred, as the DNA fragment is complementary to the probe
    - If reference (green) and unknown (red) samples both hybridise in equal proportions then the overall colour detected will be **yellow**; this shows that the gene in question is being **expressed in equal quantities** in the reference individual and the individual from whom the unknown sample was taken
    - If reference samples hybridise more than the unknown samples then the overall colour detected will be **green**; this shows that the gene is being **expressed more in the reference individual** than in the individual providing the unknown sample
    - If unknown samples hybridise more than the reference samples then the overall colour detected will be **red**; this shows that the gene is being **expressed more in the individual providing the unknown sample** than in the reference individual
    - Note that a lack of fluorescence indicates that the gene in question is **not being expressed** at all

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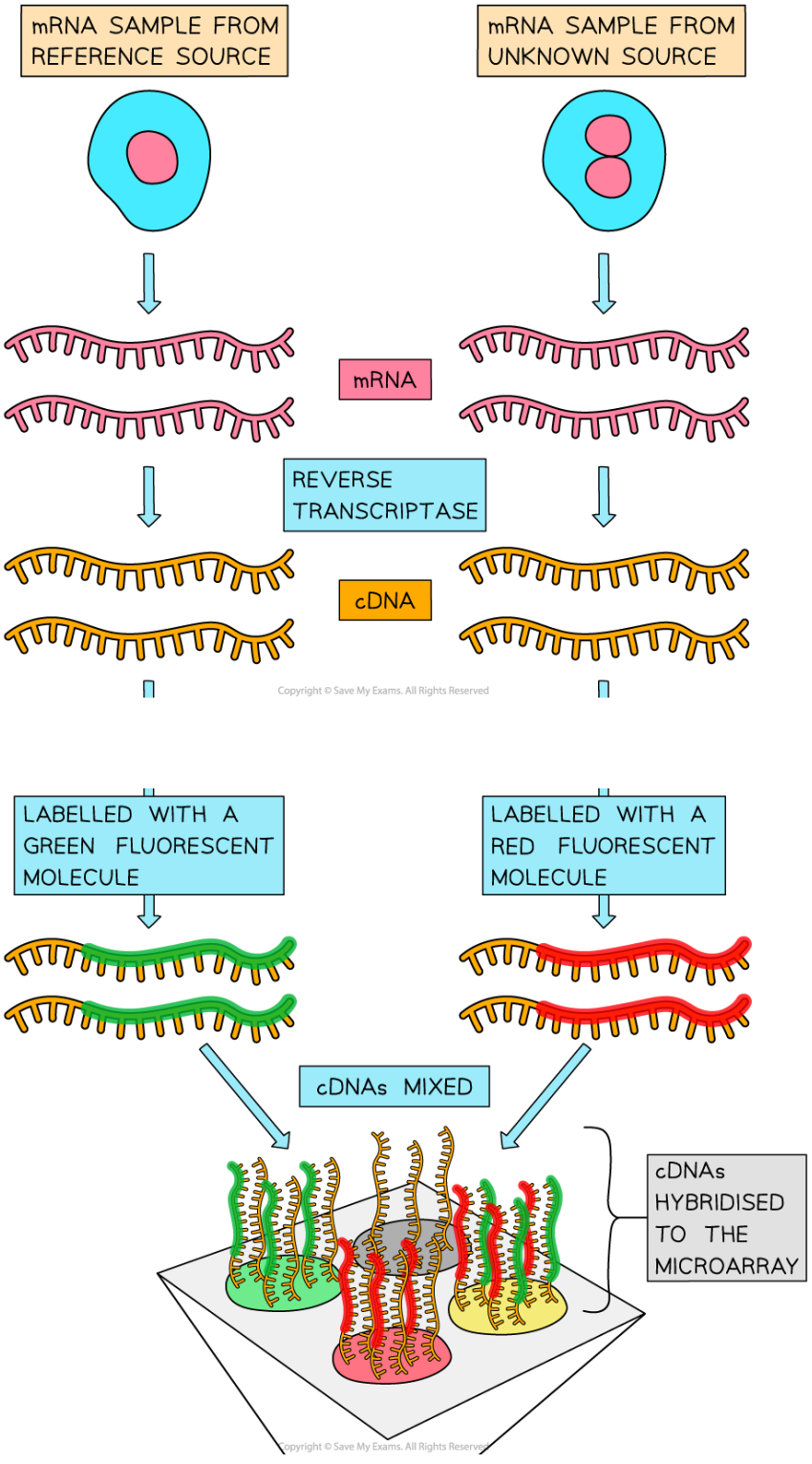
mRNA SAMPLE FROM REFERENCE SOURCE
mRNA SAMPLE FROM UNKNOWN SOURCE
mRNA
REVERSE TRANSCRIPTASE
cDNA
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LABELLED WITH A GREEN FLUORESCENT MOLECULE
LABELLED WITH A RED FLUORESCENT MOLECULE
cDNAs MIXED
cDNAs HYBRIDISED TO THE MICROARRAY
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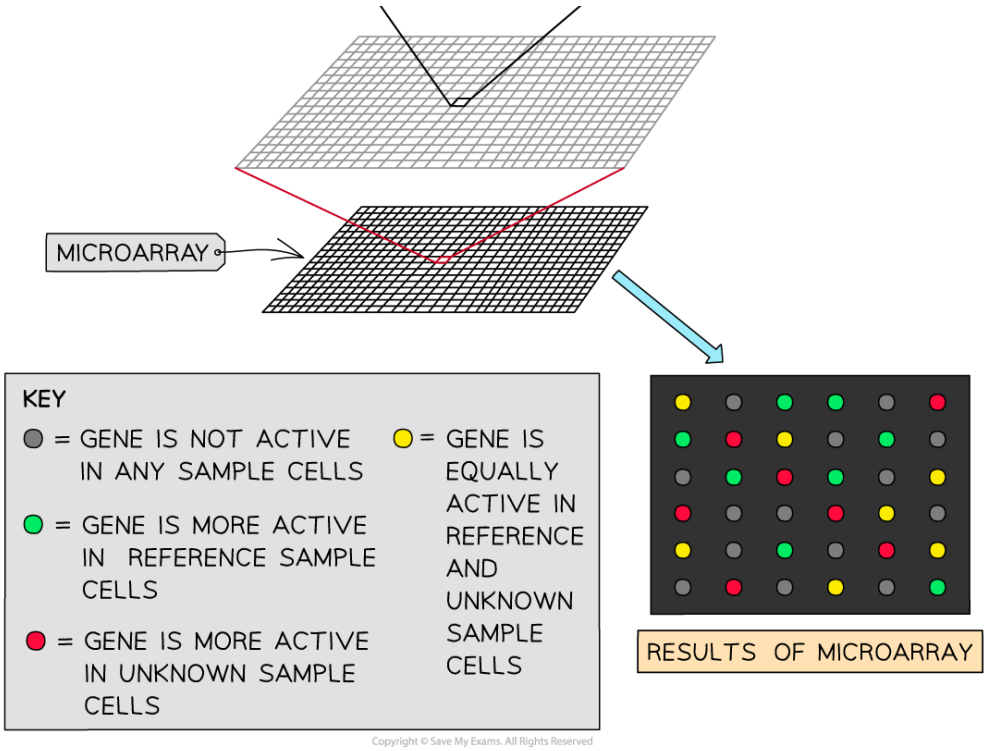

Microarrays can be used to identify active genes

- Microarrays can be used to test for **expression of genes** that increase the risk of certain **cancers**
  - E.g. High levels of expression of genes that code for **receptors that bind to the hormone oestrogen** can be a factor in the progression of some cancers; if doctors know that these genes are being expressed at high levels then **drugs that block oestrogen receptors** are likely to be an effective treatment

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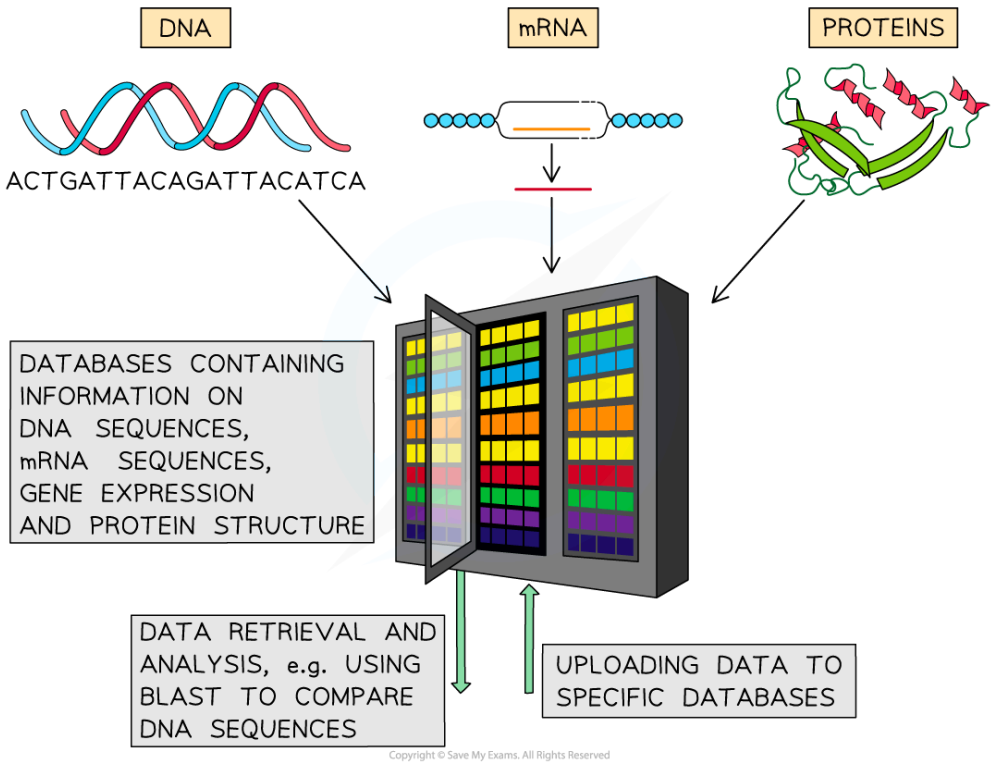
Bioinformatics

- New DNA technologies such as microarrays and sequencing generate **enormous quantities of data**, e.g.
  - Genome sequences
  - Information on gene expression
  - The amino acid sequence and functions of proteins
- To analyse all of this data scientists use **bioinformatics**
- Bioinformatics is an interdisciplinary science that incorporates **biology** with **computer technology** and **statistics** in order to collect, organise, store, and analyse **biological data**
- **Large databases** are created containing information such as **gene sequences** and **amino acid sequences** of proteins
  - The databases are available online and can perform analysis of the data selected
  - Multiple scientific research groups around the world can contribute to central databases; other groups can then analyse the research and raise queries
- There are a number of different applications of bioinformatics, e.g.
  - **Gene function** can be studied and compared across different species
  - The DNA sequences of different species can be compared and **evolutionary relationships** can be established
  - **New drug candidates** can be **identified quickly** by scanning libraries of molecules
  - Drug candidates can be **tested safely**; the impacts of different molecules on cells can be **simulated** using computer models
  - Data from microarrays and gene sequencing can be analysed to develop **personalised medical treatments** for individuals
  - New types of **genetic testing** can be developed to enable identification of genetic disease
  - Desired genes for the production of recombinant DNA can be identified, as well as determining where it is best to insert new desired genes into the genome of a genetically modified organism
    - This is useful for the development of new **gene therapies**



Bioinformatics enables the effective storage and analysis of biological data. Note that you do not need to know about BLAST analysis

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Risks & Benefits of Using GMOs

- While GMOs can be used for medical benefit, there is still **much concern about the potential impacts** of changing the genes of organisms, as well as the **ethics of genetically modifying animals**
  - These concerns are often amplified when the GMOs are **crop plants destined for human consumption**

Risks and Benefits of Genetic Engineering Table

Benefits of genetic engineering	Risks of genetic engineering
Crops can be modified to produce higher yields and have increased nutritional value, reducing famine and malnutrition	There are concerns about the long-term impacts of using genetically modified food organisms on human health
Crops can be modified to be resistant to pests and reduce pesticide use; this lowers production costs and decreases environmental damage	Some are concerned that pests may develop resistance to the modified crop defences, leading to increased used of pesticides
Enzymes used in industrial processes can be produced from genetically modified organisms; this is very cost effective	There could be transmission of genetic material between genetically modified organisms and non-GM organisms
Diseases can be treated with human proteins produced by genetically modified organisms instead of with animal proteins; this reduces the risk of allergic reactions and is more effective	Genetically modified crops are often grown in large fields, creating monocultures that are bad for biodiversity
Vaccines can be produced in genetically modified plant tissues; these do not need refrigeration, making them more accessible to people living in rural areas	Genetically modified crop varieties are usually owned by the companies that develop them, so seeds can be very expensive
Genetically modified organisms guarantee a low-cost supply of some human medications	Some have moral objections to genetically modifying animals for the sole purpose of benefiting humans