19 Genetic technology

19.1 Principles of genetic technology

a) Recombinant DNA

The aim of genetic engineering is to remove a gene (or genes) from one organism and transfer it into another so that the gene is expressed in the new host.

This DNA that has been altered is called recombinant DNA (rDNA).

- **recombinant DNA (rDNA)** – DNA made by joining pieces from two or more different sources
- **transgenic organism / genetically modified organism (GMO)** – organism that expresses the genes that have been attained from another organism because of rDNA

b) Overview of gene transfer

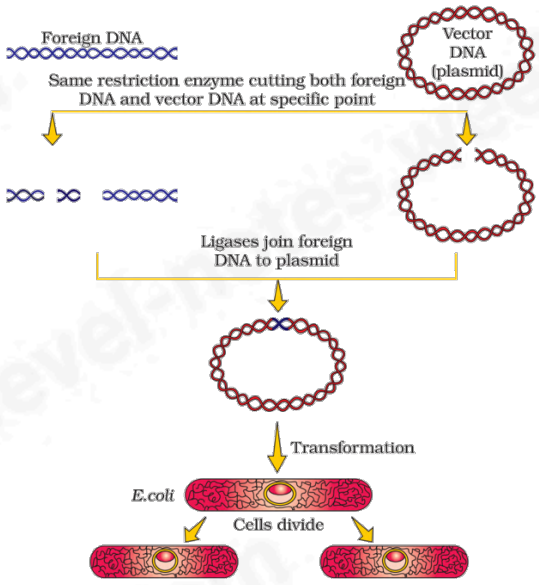


Image: http://conceptreelibrary.com/

The following steps are always necessary for the transfer of a genes into another organism *(can be of the same or different species as the source)* –

1) the desired gene is identified
2) the desired gene is isolated for it to be extracted
   - the gene can be extracted by cutting it from a chromosome using enzymes called restriction endonucleases
   - the enzyme reverse transcriptase can also be used to make a single strand of complementary DNA (cDNA) from mRNA
   - the gene can also be also be created artificially using nucleotides
3) the gene is multiplied using polymerase chain reaction (PCR)
4) the gene is inserted into a vector (e.g., plasmids, viruses, liposomes)
5) the vector delivers the gene to the cells of the organism
6) cells expressing the new genes are identified (using a marker) and are then cloned

c) Polymerase chain reaction (PCR)

PCR is an artificial method of rapidly replicating DNA under laboratory conditions, producing large quantities.

Each PCR reaction requires –

1) DNA (or RNA) sample to be amplified
2) primers
3) free nucleotides to be used in the construction of the DNA or RNA strands
4) buffer solutions to provide the optimum pH for the reactions to occur in
5) DNA polymerase

Taq polymerase

Taq polymerase is a heat-stable form of DNA polymerase extracted from a thermophilic bacterium *(Thermus aquaticus)* and is used in PCR

Features of *Taq* polymerase that enable it to be used in PCR

1) it's not destroyed in the denaturation step, so it doesn't have to be replaced each cycle
2) its high optimum temperature (72°C) means the temperature for the elongation step does not have to be dropped below that of the annealing process, so efficiency is maximised

Primers

- *Taq* polymerase can only make DNA if it's given a primer
- primers are short sequences of single-stranded DNA that have base sequences complimentary to the 3' end of the DNA or RNA being copied
- they define the region that is to be amplified by identifying to the DNA polymerase the starting point for DNA synthesis

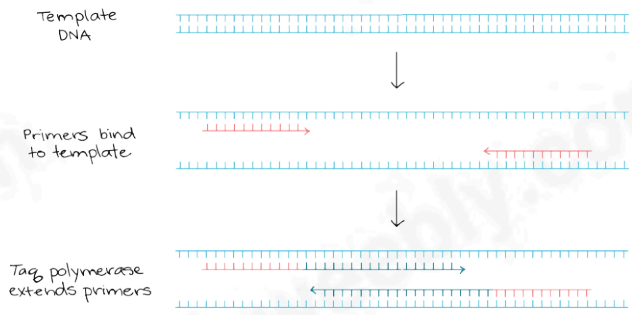
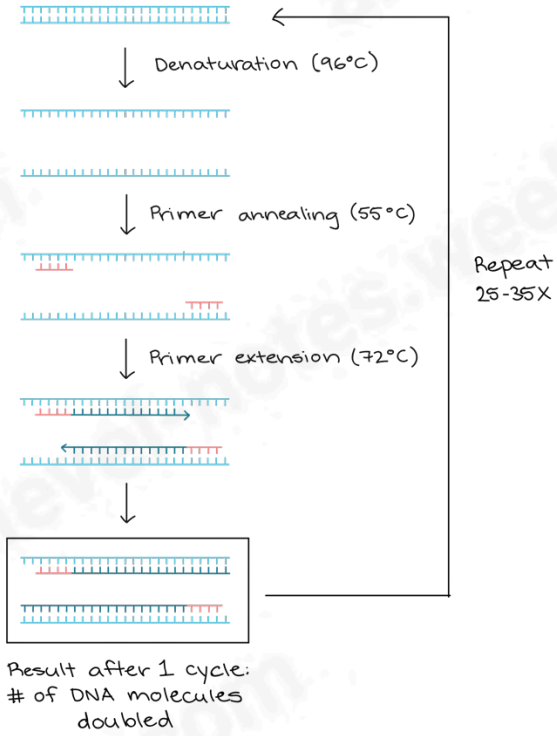


Image: https://www.khanacademy.org/

- when the primers are bound to the template, they can be extended by the polymerase and the region that lies between them will get copied

PCR can be summarised in 3 steps, and they all require different temperatures –

1) **denaturation (95°C)** – the double stranded DNA is heated to 95°C which breaks the hydrogen bonds and separates the DNA strands
2) **annealing (65°C)** – the temperature is decreased to 65°C so that primers can bind to their complementary sequences on the single-stranded template DNA
3) **elongation (72°C)** – the temperature is increased to 72°C as this is the optimum temperature for *Taq* polymerase
   - *Taq* polymerase builds the complimentary strand of DNA by extending the primer and produces new identical double-stranded DNA



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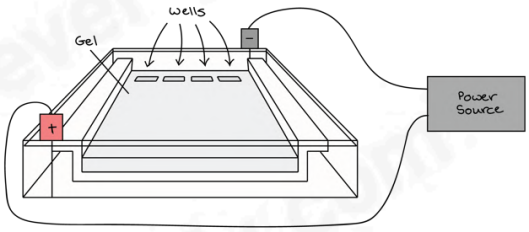
d) Gel electrophoresis

Gel electrophoresis is a technique used for separating and analysing nucleic acids or proteins based on their size and electrical charge.

1) sample is placed in well at the end of the gel
2) electric field is passed through the gel
3) negatively charged molecules are attracted to the anode
4) shorter fragments move further in unit time



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Electrophoresis of proteins

- gel electrophoresis of proteins is used to separate polypeptides produced by different alleles of the same gene e.g., the haemoglobin variants (α-globin, β-globin, and the sickle cell anaemia variant of β-globin)
- they can be separated as the different polypeptides have different net charges
- the charge on proteins is dependent on the ionisation of R groups of amino acids
- the charge of the R groups depends on the pH
- therefore, buffer solutions are used during the separation of proteins to keep the pH constant
- once electrophoresis has been performed, the results can be compared against known industry standards (e.g., from a bioinformatics database)

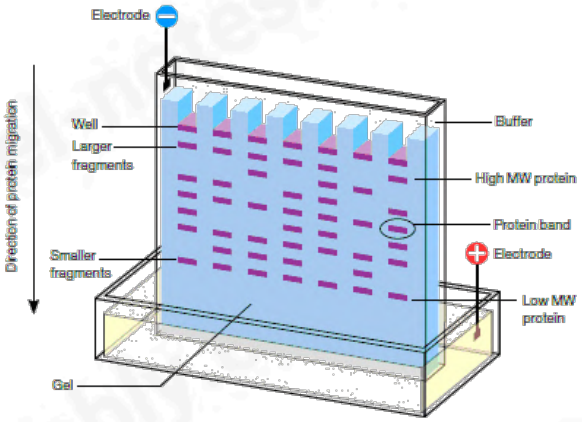


Image: https://www.bio-rad.com/

Electrophoresis of DNA

Electrophoresis of DNA is used to separate DNA fragments for DNA fingerprinting to investigate crime scenes ***(part of 19.2g)*** or to analyse genes.

These steps are for the electrophoresis of DNA for genetic profiling (fingerprinting) –

1) DNA quantity is increased using PCR
2) restriction enzymes cut the DNA into fragments (different restriction enzymes cut the DNA at different base sequences, so enzymes that will cut close to the **VNTR** regions need to be used)
3) electrophoresis is carried out on the DNA sample
4) fragments separate as DNA is negatively charged due to the presence of phosphate groups

5) all DNA fragments have the same amount of charge per mass, small fragments move through the gel faster than large ones
6) the fragments are not visible so must be transferred onto absorbent paper which is then heated to separate the two DNA strands
7) **probes** are then added, after which an X-ray image is taken, or UV-light is shone onto the paper producing a pattern of bands

If a species of animal needs to be identified from DNA electrophoresis, the band positions are compared to data on a bioinformatics database and conclusions can then be drawn.

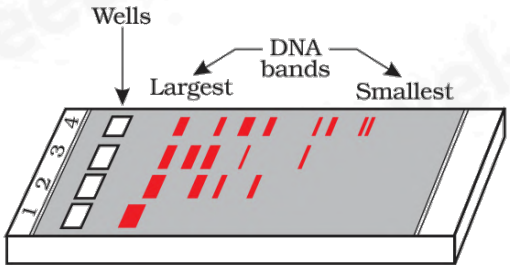


Image: http://conceptreelibrary.com/

Variable number tandem repeats (VNTR)

- these are regions of repeating sequences found in the non-coding part of DNA
- they contain variable numbers of repeated DNA sequences and vary between different people
- only identical twins share all their VNTR sequences

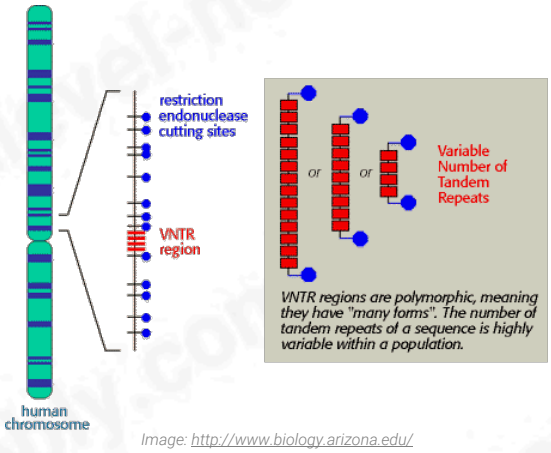


Image: http://www.biology.arizona.edu/

Probes

Probes are short sequences of single stranded DNA that have base sequences complementary to a particular VNTR region. They also contain a means by which to be identified -

1) a radioactive label (e.g., a phosphorus isotope) which causes the probes to emit radiation that makes the X-ray film go dark, creating a pattern of dark bands
2) a fluorescent stain/dye which fluoresces when exposed to UV light, creating a pattern of coloured bands

Factors affecting the movement of charged molecules in gel electrophoresis

1) **net (overall) charge** – negatively charged molecules move to anode (+), positively charged molecules move to cathode (-), highly charged molecules move faster than those with less overall charge
2) **size** – smaller molecules move faster than larger ones
3) **composition of gel** – size of pores within gel (e.g., agarose for DNA has different pore size than polyacrylamide for proteins) determines speed with which molecules move

e) Plasmids

Properties of plasmids that allow them to be used in gene cloning

1) they occur naturally in bacteria
2) can be transferred between different bacterial species
3) can be produced artificially
4) double stranded so genes from prokaryotes and eukaryotes can be inserted
5) replicate independently in bacteria

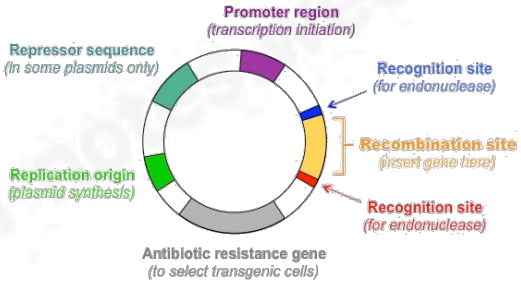


Image: https://ib.bioninja.com.au/

Transferring plasmids to host cells (bacteria)

1) the plasmids and bacteria are bathed in an ice-cold calcium chloride solution (high conc. of Ca^{2+} ions) and then heat shocked, making the bacteria's cell surface membrane more permeable
2) only a very small proportion of bacteria take up the plasmids with the gene (~1%), those that do so are said to be transformed

f) Promoters

A promoter is the region of DNA that determines which gene will be expressed as it's the region of DNA to which RNA polymerase binds as it starts transcription.

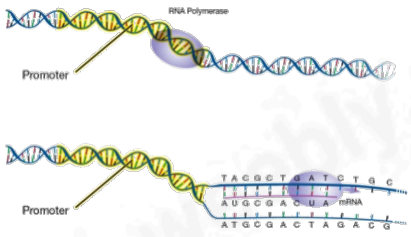


Image: https://www.genome.gov/genetics-glossary/Promoter

Role of promoters

1) ensures that RNA polymerase recognises the template strand
2) transcription start-point
3) the promoter is used to regulate gene expression because only if it is present will transcription and therefore the expression of the gene occur

Explain why promoters and other control sequences may have to be transferred as well as the desired gene

If the gene being inserted into the bacterium is to be expressed, then an appropriate promoter also needs to be inserted.

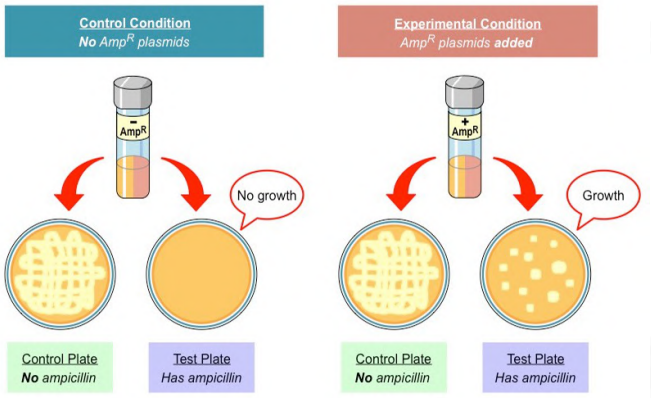
- e.g., when bacteria were first transformed to produce insulin, the gene for it was inserted next to β-galactosidase so they shared a promoter
- the promoter switched on the gene when the bacteria were in a medium with lactose but no glucose, and they produced the β-galactosidase as well as human insulin

g) Gene markers

Markers are genes coding for easily identifiable substances that can be transferred with the desired gene. They identify which cells have been successfully altered and now contain recombinant DNA.

Examples of gene markers

1) **antibiotic resistant genes** – the gene for antibiotic resistance is replaced, therefore the 'transformed' bacteria would not be able to grow in a medium with an antibiotic present



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2) **GFP (green fluorescent protein)** which fluoresces under UV light
3) **GUS (β-glucuronidase enzyme)** which transforms colourless or non-fluorescent substrates into products that are coloured or fluorescent

Disadvantages of using antibiotic resistant genes as marker genes

1) risk of antibiotic resistance genes spreading to other bacteria, producing pathogenic (disease-causing) strains that can't be killed by antibiotics
2) if the resistance spread to other bacteria this could make antibiotics less effective

Use of fluorescent genes as markers

- the GFP gene, along with the desired gene, are linked to a specific promoter
- once this promoter is activated, and the protein is expressed, the recombinant bacteria are detected when they glow green under exposure to ultraviolet light

Advantages of using of fluorescent genes as markers

1) they are easier to identify (all that is required is the ultraviolet light)
2) more economical – do not need to grow the bacteria on plates of agar infused with antibiotics
3) no risk of antibiotic resistance being passed onto other bacteria
4) there are antibiotics that are no longer effective and therefore would not stop any bacteria from growing

h) Enzymes used in genetic engineering

Three main enzymes are used – restriction enzymes (also called restriction endonuclease), ligase, and reverse transcriptase.

1) Restriction endonucleases

Restriction endonucleases are enzymes that bind to a specific target area on DNA and cuts it at the site.

Role of restriction endonucleases (restriction enzymes) in the transfer of a gene into an organism

1) isolate the desired gene

2) separate the DNA strands (at the same base sequence) in a vector so the desired gene can be inserted

Why are many different restriction endonucleases required?

- they bind to a specific restriction site (specific sequences of bases) on DNA and cuts it at that site
- e.g., the restriction enzyme *Bam*HI will cut DNA where there's a GGATCC sequence on the strand

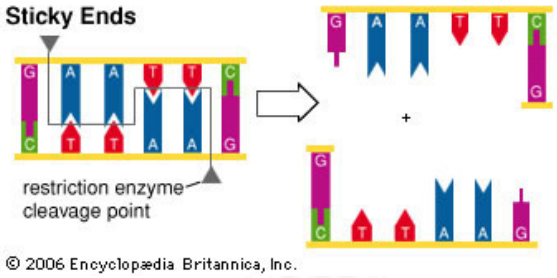
How restriction endonuclease work

- restriction enzymes cut straight across the sugar-phosphate backbone to give blunt ends
- they can also cut in a staggered fashion to give sticky ends

Sticky ends

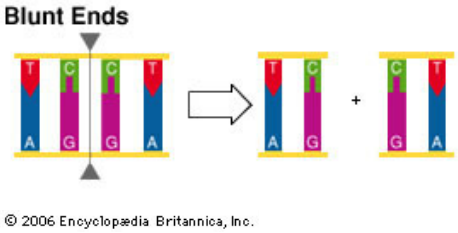
- sticky ends are short lengths of unpaired bases
- they make it easier to insert the desired gene into another organism's DNA or into a vector as they can easily form hydrogen bonds with complementary

sequences of bases on other pieces of DNA cut with the same restriction enzyme



Blunt ends

Blunt ends are fragment ends of a DNA molecule that are fully base paired.

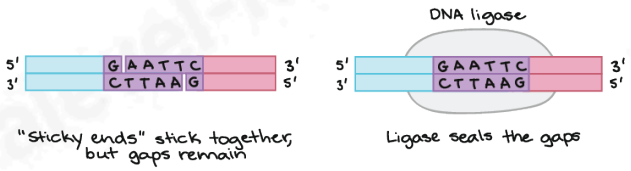


2) (DNA) ligase

Ligase catalyses the formation of phosphodiester bonds in the DNA sugar-phosphate backbone.

Role of ligase in the transfer of a gene into an organism

Enables the isolated desired gene to be spliced into a vector (generally a plasmid) so that it can be transferred to the new organism.



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3) Reverse transcriptase

- reverse transcriptase is an enzyme encoded by retroviruses that uses an RNA strand as a template for DNA synthesis (produces 1 DNA strand)
- this DNA that's been synthesised is called complementary DNA (cDNA)
- the source of reverse transcriptase enzyme is retroviruses

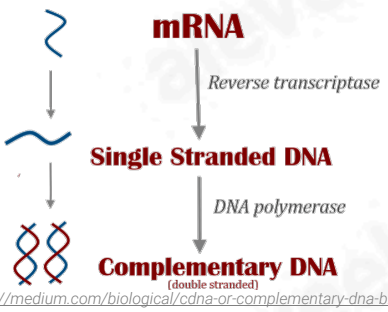


Image: https://medium.com/biological/cdna-or-complementary-dna-b283d9d86c1f

Role of reverse transcriptase in the transfer of a gene into an organism

- produce a single-strand complementary DNA molecule (cDNA) that contains the code for the desired characteristic
- this will then be inserted into a vector (after being converted into a double-stranded DNA molecule

Advantage of using reverse transcriptase enzymes

- easier for scientists to find mRNA with the specific characteristic
- this is because specialised cells make very specific types of mRNA (e.g., β-cells of the pancreas produce many insulin mRNA)
- mRNA also does not contain introns
  - introns are non-coding regions of RNA transcript

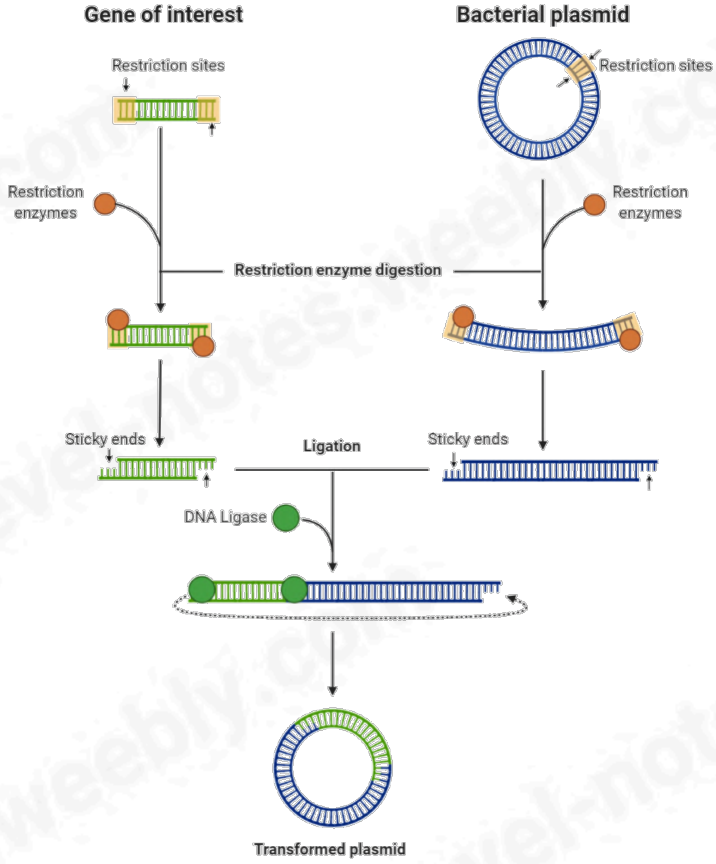


Image: https://app.biorender.com/

i) Microarrays (DNA chips)

- microarrays are laboratory tools used to
  - identify the genes present in an organism's genome
  - find out which genes are expressed within cells
  - compare the genes present in 2 different species

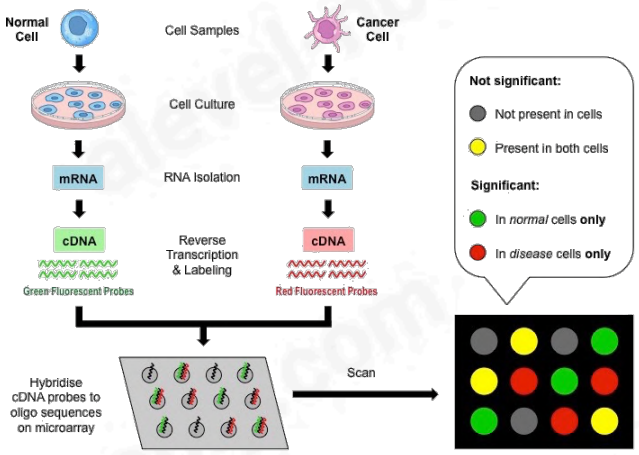


Image: https://ib.bioninja.com.au/

- a microarray consists of a small (usually 2cm²) piece of glass, plastic, or silicon (also known as chips) that have probes attached to a spot (called a gene spot) in a grid pattern
  - each probe represents a known gene sequence
  - if a gene is active within a cell, then the cDNA (produced from the mRNA transcript) will bind to its complementary probe

How microarrays are used in the analysis of genomes

1) DNA is collected from the species that are going to be compared
2) restriction enzymes are used to cut the DNA into fragments
3) the fragments are denatured to form single-stranded DNA molecules (cDNA)
4) the DNA is labelled with fluorescent tags (the fragments from the different sources are tagged different colours, usually red and green)
5) the labelled DNA samples are mixed together and allowed to hybridise with the probes on the microarray
6) any DNA that has not bound to the probes is washed off
7) the microarray is inspected using UV light, causing the tags to fluoresce
8) the presence of colour indicates that hybridisation has taken place (as the DNA fragments are complementary to the probes)

‣ red and green: DNA from one species has hybridised with probes

‣ yellow: DNA from both species hybridised (the two species have DNA with exactly the same base sequence)

‣ no colour/blue: no hybridisation, gene not present in either species

9) the microarray is scanned so data is read by a computer and stored

How microarrays are used in detecting mRNA in studies of gene expression

Microarrays are used to compare which genes are active by identifying the genes that are being transcribed onto DNA.

1) mRNA is collected from 2 types of cells and reverse transcriptase is used to convert mRNA to cDNA
2) PCR may be used to increase quantity of cDNA as mRNA quantity is quite low at any one time
3) cDNA is labelled with fluorescent tags and denatured to give single stranded DNA
4) single stranded DNA is allowed to hybridise with probes on the microarray
5) UV light is shone; spots that fluoresce indicate the genes that were being transcribed in the cell

‣ intensity of light emitted by each spot indicates the level of activity by each gene

‣ high intensity: indicates many mRNA molecules are present in sample

19.2 Genetic technology applied to medicine

a, b) Bioinformatics

- **bioinformatics** – the collection, processing, and analysis of biological information and data using computer software

The role of bioinformatics following the sequencing of genomes

1) comparisons can be made with other known genomes using the many databases available – sequences can be matched, and degrees of similarity calculated
2) human genes such as those associated with development can be found in other organisms e.g., Drosophila; ∴ Drosophila could be used in experiments as a model for humans
3) ways to control Plasmodium and gene sequencing is helping in the development of vaccines for malaria

c) Producing human proteins by recombinant DNA techniques

Genetic technology allows products specific to humans to be made. Human proteins produced by DNA recombinant techniques include –

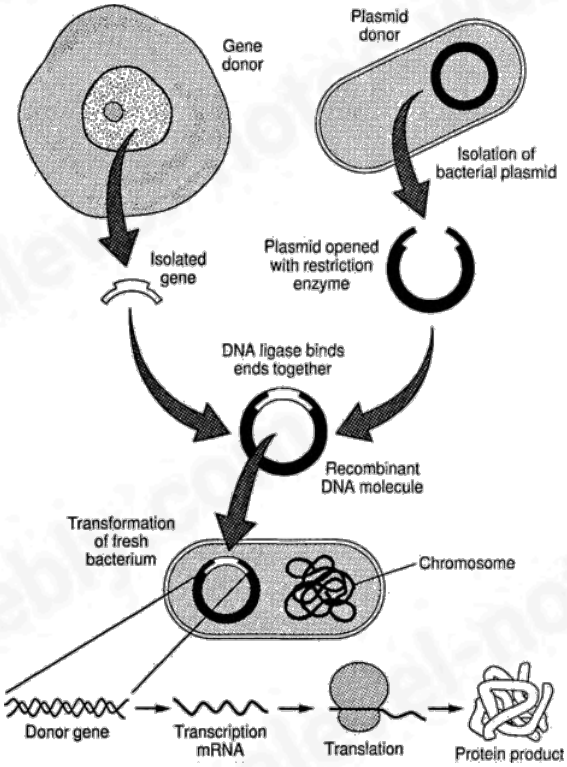
a) insulin
b) factor VIII (for the treatment of haemophilia)
c) adenosine deaminase (for the treatment of SCID)

Bacteria, yeasts, and cultures of mammalian cells can be used to produce these proteins.

advantages of producing human proteins by recombinant DNA techniques
1) these cells have simple nutritional requirements
2) large volumes of product are produced
3) production facilities do not require much space and the processes can be carried out anywhere in the world; reliable supply available
4) few practical and ethical problems as proteins do not have to be extracted from animal sources
5) the proteins are engineered to be identical to human proteins or have modifications that are beneficial

Why are most recombinant human proteins are produced using eukaryotic cells (e.g., yeast or mammalian cells in culture) rather than using prokaryotic cells?

- eukaryotic cells will carry out the post-translational modification (due to presence of Golgi Apparatus / enzymes)
- this is required to produce a suitable human protein



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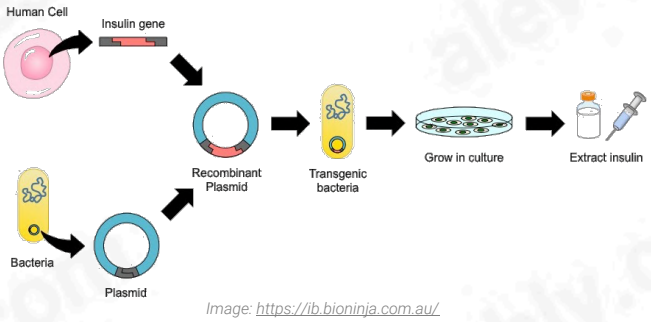
Producing recombinant insulin

1) bacteria plasmids are modified to include the human insulin gene
   - restriction endonucleases are used to cut open plasmids
   - DNA ligase is used to splice the plasmid and human DNA together
2) recombinant plasmids are then inserted into Escherichia coli, transforming the cells
3) once the transgenic bacteria are identified (by the markers), they are isolated, purified and placed into fermenters that provide optimal conditions
4) they multiply by binary fission, and express the human protein - insulin, which is eventually extracted and purified



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Advantages of using recombinant insulin

1) identical to human insulin, unless modified to have different properties (e.g., act faster, which is useful for taking immediately after a meal or to act more slowly)
2) there is a reliable supply available to meet demand (no need to depend on availability of meat stock)
3) fewer ethical, moral, or religious concerns (proteins are not extracted from cows or pigs)
4) fewer rejection problems or side effects or allergic reactions
5) cheaper to produce in large volumes
6) useful for diabetics who have animal insulin tolerance

Producing recombinant factor VIII

Factor VIII is a blood-clotting protein that haemophiliacs cannot produce

1) kidney and ovary hamster cells have been genetically modified to produce factor VIII
2) once modified, the cells are cultured in fermenters
3) due to the optimal conditions in the fermenter, the hamster cells constantly express factor VIII which can then be extracted and purified
4) the product is used as an injectable treatment for haemophilia

Advantages of using recombinant factor VIII

1) fewer ethical, moral, or religious concerns (proteins are not extracted from human blood)

2) less risk of transmitting infection (e.g., HIV) or disease
3) greater production rate

Producing recombinant adenosine deaminase (ADA)

Severe combined immunodeficiency (SCID)

- a crippled immune system due to the inability to make adenosine deaminase (ADA)
- sufferers may die at infancy due to normal infections
- T-lymphocytes of sufferers are removed, and normal alleles of the ADA gene are introduced into them using a virus vector however this is not a permanent cure

The enzyme adenosine deaminase is used to treat severe combined immunodeficiency (SCID) while patients are waiting for gene therapy or when gene therapy is not possible.

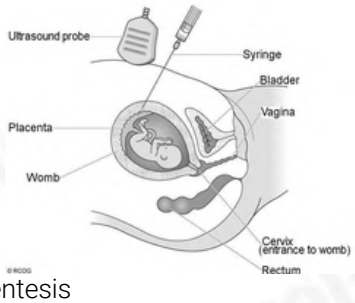
- the larva of the cabbage looper moth has been genetically modified (using a virus vector) to produce ADA

d) Genetic screening

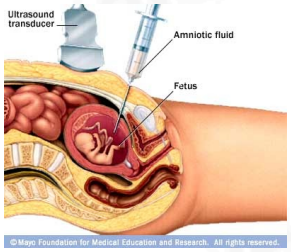
- this the analysis of a person's DNA to check for the presence of a particular alleles
- can be done in adults, an in vitro embryo, or an embryo or foetus in a uterus

How a sample of DNA to be analysed can be obtained (from adult, embryo, foetus)

1) taking tissue samples from adults or embryos produced by in-vitro fertilisation
2) chorionic villus sampling
   - a small sample of part of the placenta called the chorion is removed by a needle



3) amniocentesis
   - needle puncture of the amniotic sac to withdraw amniotic fluid for analysis



Genetic screening for the faulty alleles of *BRCA1* and *BRCA2*

- *BRCA1* and *BRCA2* are genes that produce tumour suppressor proteins and thus they play an important role in regulating cell growth
- faulty alleles of these particular genes exist (can be inherited from either parent
- inheritance of these faulty alleles increases the risk of the individual developing breast cancer

Advantages of genetic screening

1) preventative measures can be taken e.g., an elective mastectomy (breast removal) to reduce the risk of developing cancer
2) screening for breast cancer may begin from an earlier age or more frequently
3) enables the person to participate in research and clinical trials

Genetic screening for haemophilia

Haemophilia is a sex-linked recessive inherited disease where the body does not produce a blood protein (either factor VIII or factor IX) that is required for the blood to clot.

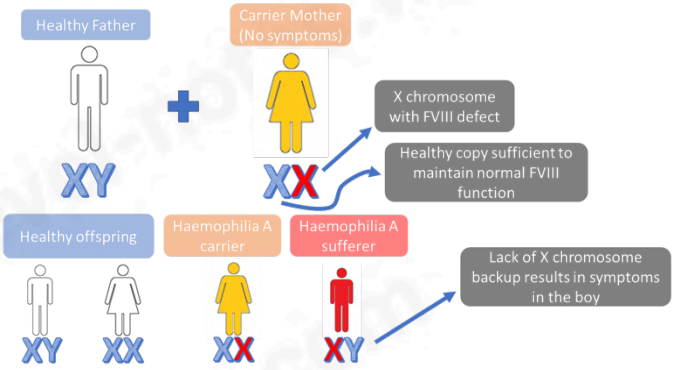


Image: https://blogs.surrey.ac.uk/

Advantages of genetic screening for women

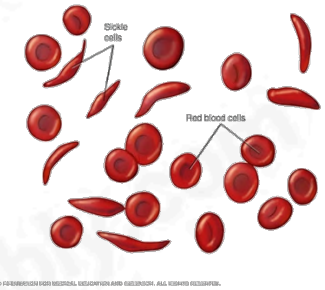
1) can determine whether they are carriers or not (as haemophilia is a recessive disease)
2) can help the women (and their partners) make decisions about future pregnancies (if they're carriers)
3) help the doctors take special precautions during the pregnancy (if a carrier)
4) women can use pre-implantation genetic diagnosis (PGD) during IVF to choose an embryo that is carrying the allele for the relevant blood clotting factor as opposed to embryos carrying the recessive alleles

Genetic screening for sickle cell anaemia

- sickle cell anaemia is an autosomal recessive disease that results in the haemoglobin molecule

being less soluble if oxygen is not present, causing red blood cells to form a sickled shape

- this sickled shape reduces the ability of the red blood cell to carry oxygen, the cells are less flexible, and more prone to getting stuck in small capillaries



Advantages of genetic screening

1) people with a family history of the disease or African ancestry may get tested to determine if they are a carrier
2) if the person is determined to be a carrier, they can discuss with a genetic counsellor their options so they can make informed decision
3) if the person is undergoing IVF, they could use PGD to select an embryo that does not have the recessive alleles

Genetic screening for Huntington's disease

Huntington's disease is a late-onset neurodegenerative disease caused by an autosomal dominant allele.

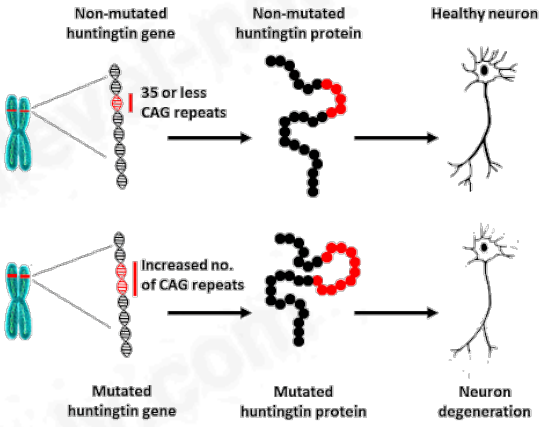


Image: http://www.ehdn.org/about-hd/

Symptoms present when the person is middle-aged, by which time they might've already had children.

There is no cure, and the treatments available only alleviate symptoms.

Advantages of genetic screening

1) people can plan for the future (how they will live and be cared for)
2) couples can make informed reproductive decisions (as the risk that their children may inherit the disease is 50%)
3) people to participate in research and clinical trials

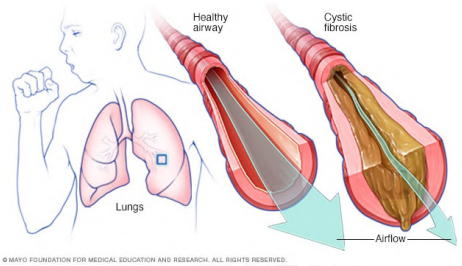
People in families with Huntington's face a dilemma –

- would they be told they're at high risk for developing the disease, although nothing can ever be done about it?
- would they live with the uncertainty of not knowing?
- some people with the dominant allele for Huntington's may live their whole life completely free of the disorder as it sometimes does not develop

Genetic screening for cystic fibrosis

Cystic fibrosis is an autosomal recessive genetic disorder that is caused by a deletion mutation of 3 bases (AAA) of the gene that codes for a transporter protein called CFTR.

It is a progressive disease in which abnormally thick mucus is produced in the lungs and other parts of the body (pancreatic duct, ducts in the reproductive system).



- CFTR sits in the cell surface membranes of cells and allow chloride ions to pass out of the cells
  - the high concentration of chloride ions outside cells reduces the water potential causing water to flow out via osmosis
  - this water mixes with the mucus outside, making it easier for cilia to remove it
- in cystic fibrosis, the faulty CFTR protein no longer transports chloride ions across the cell surface membrane and therefore water does not move by osmosis across the membrane
- there is no cure for cystic fibrosis, although there are many different treatments that help alleviate symptoms

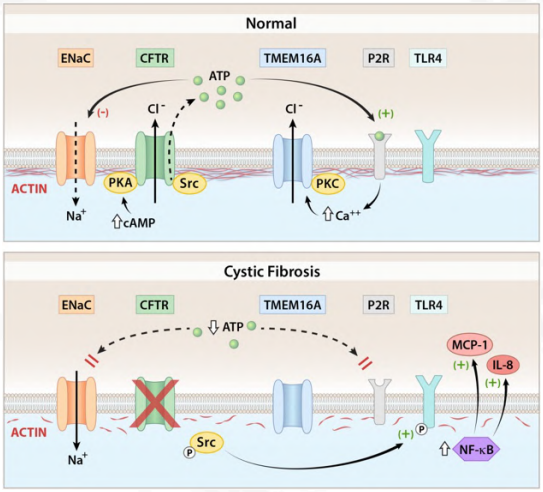


Image: https://aasldpubs.onlinelibrary.wiley.com/

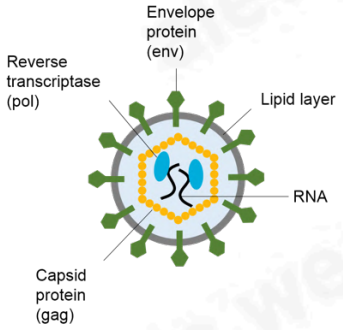
e) Gene therapy

Gene therapy is the insertion of 'normal' alleles of a gene into the cells of a person with a genetic disorder in an attempt to cure the disorder.

The most common vectors that are used to carry normal alleles to host cells in gene therapy include –

1) viruses
   - retrovirus
   - lentivirus
   - adeno-associated virus (AAV)
2) liposomes
3) naked DNA (used sometimes)

Viruses in gene therapy

	1. RETROVIRUS
features	 Image: http://2014.igem.org/ • viruses that contain two copies of single-stranded RNA as their genetic material • in a host cell, it uses the enzyme reverse transcriptase to translate its genetic information into DNA • this DNA can integrate into the host genome - mRNA can then be made by transcription of the viral DNA
in gene therapy	• retroviruses insert their genes into the host's genome however do so randomly • this means they may insert their genes within another gene or into the regulatory sequence of a gene (which may then activate a nearby gene causing cancer)
example	HIV (human immunodeficiency virus)

	2. LENTIVIRUS
features	• lentiviruses are a subtype of retroviruses • these are viruses characterised by having long incubation periods
in gene therapy	• lentiviruses insert genes randomly into the host's genome • however, this virus can be modified to not replicate
example	HIV (human immunodeficiency virus)

	3. ADENO-ASSOCIATED VIRUS
features	• small, non-enveloped virus which has single stranded DNA as its genetic material
in gene therapy	• this virus does not insert its genes into the host genome and so they aren't passed onto daughter cells when they divide • this is a problem when cells are short-lived such as lymphocytes • however, they have been used successfully with long-lived cells such as liver cells and neurones

Liposomes in gene therapy

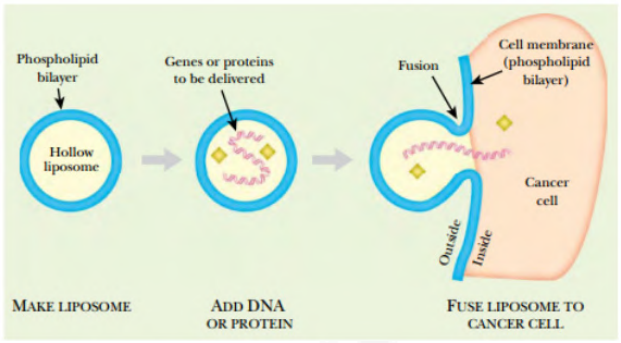


Image: https://istudy.pk/liposomes-lipoprofection-gene-therapy/

- liposomes have been used in curing cystic fibrosis by gene therapy
- the normal allele for the CFTR gene is inserted into liposomes
- this is then was sprayed as an aerosol into the noses of volunteers
- this succeeded in the allele into a few cells lining the nose, however the effects only lasted a few weeks as these cells have a very short natural lifespan

Naked DNA in gene therapy

- DNA has been inserted directly into tissues without the use of any vector
- naked DNA has been using in trials of gene therapy for skin, muscular, and heart disorders
- advantage of using naked DNA in gene therapy – removes problems associated with using vector

Genetic diseases that have been treated using gene therapy

1) Leber congenital amaurosis

- a form of hereditary blindness caused by retinal cells dying off gradually from a young age in males
- doctors injected adeno-associated viruses into the retina which contain the normal alleles of one of the genes that caused damage to the photoreceptors, improving their eyesight

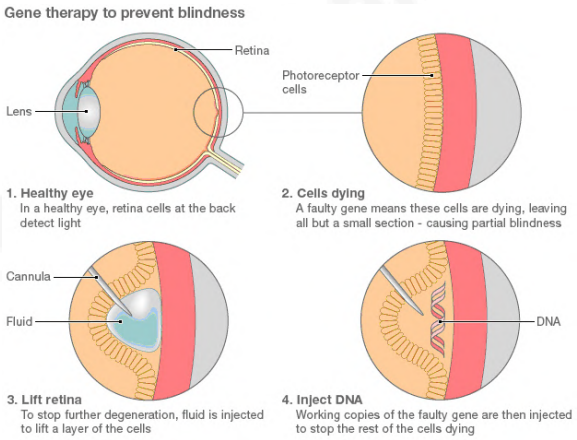


Image: https://www.retinacv.es/tag/celulas/

2) SCID

- T-lymphocytes were removed from the patient and normal alleles of ADA gene were introduced into them
- a viral vector was used
- cells were replaced
- this cure was not permanent – regular transfusions were necessary to keep the immune system functioning

Somatic and germ cell therapy

The gene therapies described above have been carried out in body cells (somatic cells).

Another possibility is to insert alleles into germ cells – i.e., cells that are involved in sexual reproduction (gametes or early embryo).

- **germ cell gene therapy** – attempts to alter alleles in cells involved in sexual reproduction

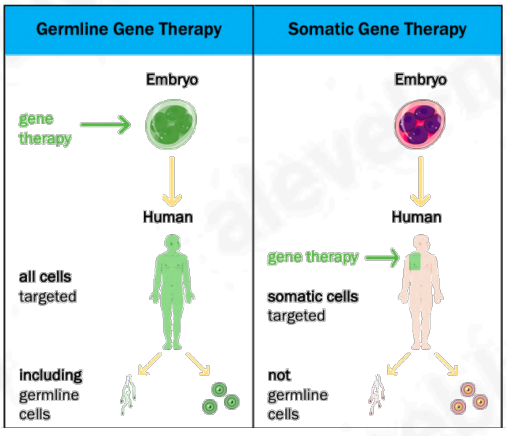


Image: https://the-gist.org/2019/05/embryo-gene-editing-changing-life-as-we-know-it/

- e.g., a woman with cystic fibrosis could opt to try conceiving a baby using IVF
  - eggs would be harvest from her in the normal way
  - the 'correct' allele of the CFTR gene could be injected into an egg and this egg fertilised to produce a zygote

f) Social and ethical considerations of using gene therapy

1) the potential for side effects that could cause death (e.g., the children who were treated for SCID developed leukaemia)
2) whether germ cell gene therapy should be allowed
3) germline therapy is controversial as the change can be passed on to the children of the treated person and all subsequent generations
4) should gene therapy be used in treating genetic conditions where treatments already exist
5) the expense of treatments as multiple injections of the genes may be required if the somatic cells are short-lived; this may make the cost of gene therapy accessible to a limited number of people
6) who has the right to determine which genes can be altered and which cannot?

g) PCR and DNA testing in forensic medicine and criminal investigations

See 19.1d, "Electrophoresis of DNA" for more details.

1) electrophoresis of DNA is used in genetic profiling (fingerprinting) in forensic science
2) PCR is used in forensic science to solve crimes: used to amplify DNA from small tissue samples

19.3 Genetically modified organisms in agriculture

The ability to manipulate genes has many potential benefits in agriculture, but the implications of releasing genetically modified organisms (GMOs) into the environment are subject to much public debate in some countries.

a) The significance of genetic engineering in improving the quality and yield of crop plants and livestock in solving the demand for food

Crop plants have been genetically modified to be –

1) **resistant to herbicides** – increases productivity / yield
2) **resistant to pests** – increases productivity / yield (e.g., Bt maize)
3) **enriched with vitamins** – increases the nutritional value (e.g., Golden rice™)

Bt maize

1) maize has been genetically modified with a gene for Bt toxin
2) gene for Bt toxin is taken from the bacterium *Bacillus thuringiensis*
3) Bt toxin is lethal to insects that eat it but harmless to other animals
4) genetically modified crop plants with Bt toxin gene produce their own insecticides
5) maize is protected against corn borers
6) Bt resistance in corn borers is a recessive allele; adult corn borers in refuges (non-GM maize) are homozygous dominant or heterozygous and supply the dominant alleles to counteract the resistance when adult corn borers from fields and refuges mate

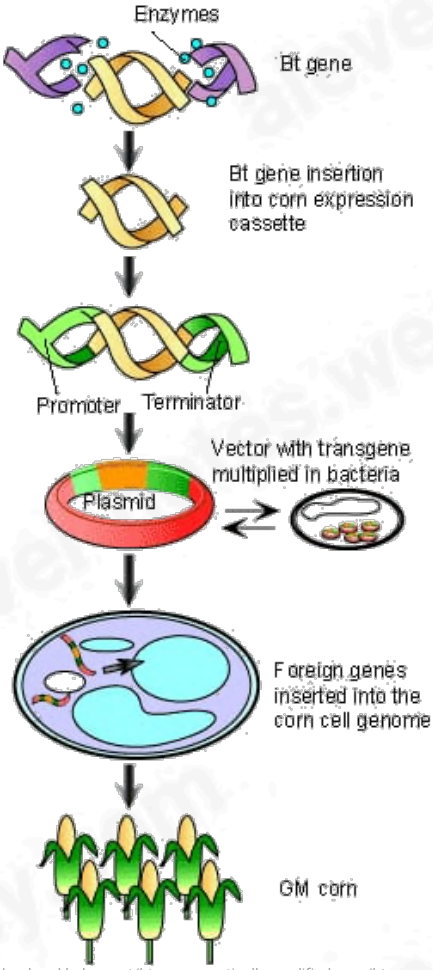


Image: https://schoolworkhelper.net/bt-corn-genetically-modified-corn/bt-corn-genetically-modified-corn/

Vitamin A enhanced rice (Golden rice™)

- vitamin A is a fat-soluble vitamin found in oily fish and animal products such as eggs, milk, cheese & liver
- also made in human bodies from carotene (orange carotenoid pigment)
- deficiency can cause blindness and immune deficiency syndrome

Q) Describe how the vitamin A content of rice can be enhanced by genetic modification. [9 marks]

1) vitamin A is found in the aleurone layer of rice
2) white rice does not contain the aleurone layer
3) genes that code for vitamin A are extracted from
4) bacteria *(Pantoea ananatis)*
5) and daffodils
6) the genes are inserted into plasmids
7) and promoters are added
8) the plasmids are put into *Agrobacterium tumefaciens*
9) *Agrobacterium tumefaciens* is mixed with rice embryos
10) some embryos take up bacteria and the vitamin A gene
11) the plants grow into adult plants
12) and produce seeds with vitamin A
13) in the endosperm
14) this variety of rice is called Golden Rice™

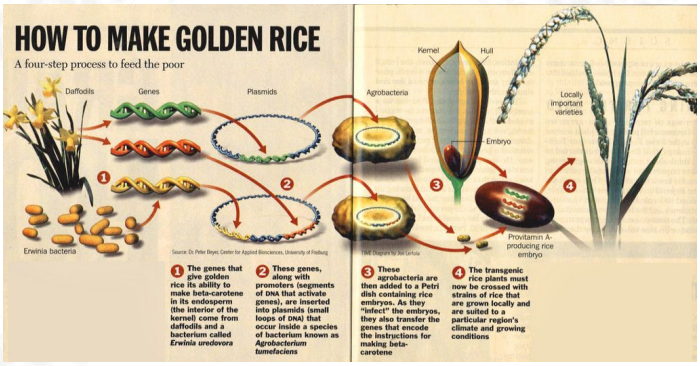


Image: https://cellsandbeyond.wordpress.com/

Ethical implications of Golden Rice™

- some organisations condemn Golden Rice™ saying it is the wrong way to solve poverty
- solving political, cultural, and economic issues will help lower poverty, and people can afford to have a more varied diet

GM Salmon

1) growth-hormone regulating genes from Pacific Chinook salmons and promoters from ocean pout are injected into the fertilised egg of an Atlantic salmon
2) this enables the salmon to produce growth hormone throughout the year and therefore grow all year instead of just the summer and spring
3) they reach market size in 18 months as opposed to 3 years of an unmodified fish
4) to prevent the GM salmon from reproducing in the wild, all the salmon are female and sterile

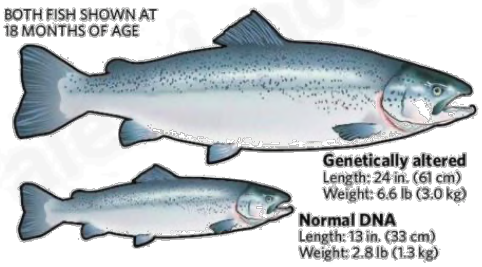


Image: https://seafood.media/

Benefits of using genetic engineering rather than traditional selective breeding techniques to solve the global demand for food

1) organisms with the desired characteristics are produced more quickly
2) all organisms will contain the desired characteristic (there is no chance that recessive allele may arise in the population)
3) the desired characteristic may come from a different species / kingdom

Consequences of using genetically engineered organisms to solve the global demand for food

1) the development of resistance for the genes that have been introduced
2) the risk of the gene spreading to wild relatives
3) the modified organism may become a pest
4) the reduction in biodiversity
5) potential ecological effects (e.g., harm to non-targeted species)

b) Increasing crop production by using varieties that are herbicide and insect resistant

Cotton

Genetically modified cotton has been protected against boll weevil.

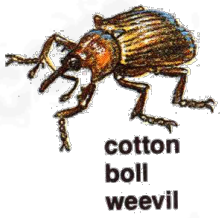


Image: https://farm4.staticflickr.com/3210/2523859654_d16a0afbc9.jpg

Tobacco

- tobacco has been made resistant to 2 different herbicides (sulfonylurea & dinitroaniline)
- in both cases the genes were taken from other species of plant
- tobacco has also been made insect-resistant against the tobacco bud worm

Oil seed rape

1) a source of vegetable oil which is used as a biodiesel fuel and lubricant
2) modified oil seed rape is resistant to the herbicide glyphosate (inhibits the synthesis of 3 amino acids: phenylamine, tyrosine, tryptophan which are required for producing essential proteins) and contains lower concentrations of erucic acid glucosinolates
3) the gene transferred into crop plants came from a strain of the bacterium *Agrobacterium*

Detrimental effects on the environment of growing a herbicide-resistant crop

1) the genetically modified plant will become an agricultural weed
2) pollen will transfer the gene to wild relatives, producing hybrid offspring that are invasive weeds
3) herbicide-resistant weeds will evolve because so much of the same herbicide is used

Detrimental effects on the environment of growing an insect-resistant crop

1) the evolution of resistance by the insect pests
2) a damaging effect on other species of insects (however, less pesticide is used, less risk of spray carrying and affecting non-target species in other areas)
3) the transfer of added gene to other species of plant

Disadvantages of using genetically engineered organisms to increase the productivity of the crop

1) the development of resistance for the genes that have been introduced
2) the risk of the gene spreading to wild relatives
3) the modified organism may become a pest
4) the reduction in biodiversity
5) potential ecological effects (e.g., harm to non-targeted species)
6) possible risk to human health as an allergy (there are no long-term studies on the effect on human health)

c) Social and ethical implications of using genetically modified organisms (GMOs) in food production

1) modified crop plants may become agricultural weeds or invade natural habitats
2) the introduced genes may be transferred by pollen to wild relatives whose hybrid offspring may become more invasive
3) the introduced genes may be transferred by pollen to unmodified plants growing on a farm with organic certification

4) the modified plants may be a direct hazard to other animals and humans by being toxic or producing allergies
5) the herbicide that can now be used leaves toxic resides on the crop
6) genetically modified seeds and herbicides are expensive, and their cost removes any advantage of growing a resistant crop
7) growers need to buy new seeds every season, keeping costs high
8) danger of losing traditional varieties with their desirable background genes and possibly unknown traits which might be useful