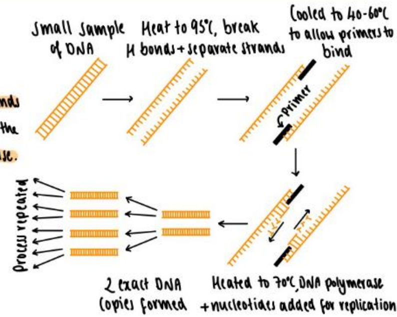
gene technology

Polymerase Chain Reaction

> A technique used to amplify (make many copies of) a sample of DNA



1. DNA section to be amplified, heated to 95°C, breaks H bonds + separates strands
2. Add primers (short chains ~20 nucleotides long, which are comp to the bases in the part of the DNA strand selected), nucleotides (ATCG) + enzyme DNA polymerase.

Primers:
- Stop the 2 DNA strands rejoining
- 'Bracket' the DNA section to be copied
- DNA rep. can only start in a double-stranded region

3. DNA cooled to 40-60°C to allow the primers to anneal to their complementary sequences on the separated DNA strands.
4. Heated to ~70°C to allow the DNA polymerase enzyme to replicate the rest of the DNA
   ↳ extends primers

Taq polymerase: → heat stable DNA polymerase
- Thermostable enzyme from thermophilic bacterium Thermus aquaticus
- Not denatured ⇒ 70° = optimum

5. Each orig DNA mol. replicated to form 2 mol.
   ↳ cycle repeated ~25 times with the no. of DNA mol. doubling in each cycle
   ↳ 25 cycles produces millions of copies of the original DNA in a very short space of time

PCR can be completely automated, so in a few hours a tiny sample of DNA can be amplified millions of times with little effort

Any contaminent DNA will also be amplified
↳ can cause problems eg. in court cases

Uses of PCR:
- Resolve paternity disputes
- Check immigration applications
- Confirm animal pedigrees
- Forensic medicine → can use minute samples of DNA, which may be taken from samples of blood, hair or semen
- Copy DNA from extinct organisms → identify relationships with living species
- Genetic screening + research involving genetic diseases

DNA (Gene) Probes

> A short length of DNA with a known base seq.
> Locates a specific section of DNA / gene

A A A T G C C A G G G T A T C G C T T ← DNA section
↓ treatment with restriction endonuclease
AAATG, GGGTATC, GCTT, CCA } DNA fragments
fluorescently labelled DNA probe added
DNA fragments separate based on size (length)
well
gel electrophoresis → nylon mem. → uv light
fluorescent band
electric current through gel, smallest move farthest
shows if it attaches

- target DNA is hydrolysed by restriction endonucleases + DNA fragments separated by gel electrophoresis
- DNA sections transferred to nylon membrane + DNA probe is added
- Consists of a short single strand of DNA (eg. 20 nucleotides in length) that contains the known sequence of bases.
- Labelled → radioactive (eg. 5' end labelled with ^{32}P) - detected by x-ray film
  → fluorescent - detected by UV light
- Bases in DNA probe combine (hybridise) with the complementary bases on the donor DNA, revealing the position of the gene.
- Once located the gene can be extracted

> Any DNA probes that have not attached need to be 'washed off' as they would still show up.

> Gel electrophoresis separates the DNA sections based on size (length), with shorter sections of DNA travelling faster + further through the gel

Nucleotide Sequences + Genetic Markers

› Differences in nucleotide sequences can be identified by:
- gene sequencing + working out the order of the bases → measure of genetic variation
- using genetic markers eg. a section of DNA may act as a marker for a particular disease
  ↳ genetic screening involves the identification of a particular DNA sequence which indicates that someone has a particular disease

2 types of marker

1.) › Microsatellite repeat sequences (MRSs)
- Sections of non-coding DNA where a small no. of bases (eg. 2 or 3) are repeated many times → ATC ATC ATC ATC ATC
- No. of repeats (also called short tandem repeats - STRs) unique to each individual
- The uniqueness of MRSs in each individual forms the basis of DNA (genetic fingerprinting)

2.) › Single nucleotide polymorphisms (SNPs)
- Differences in single ucleotides among samples of DNA molecules eg. DNA sequence could be AAGCCTA in one individual and AAGCTTA in other
- SNPs may indicate the cause of a genetic disease eg. sickle-cell anaemia is caused by a change in a single base in the DNA sequence

Microarray Technology

microarrays currently less expensive than seq.

› miniature spotting tile with thousands of 'spots' (DNA probes → each diff. with a unique base seq.) on it.
↳ involves DNA bound (hybridised to a microarray chip
› enables rapid gene expression profiling or gene seq. variation of thousands of genes in an individual

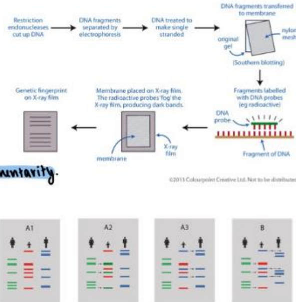
Uses: → investigate drug responses
→ investigate disease alleles
→ forensic analysis

› Gene sequences → DNA amplified by PCR, cut by restriction enzymes, made single-stranded + a label is added (label → fluorescent/chemiluminescent)
↳ added to the microarray
→ only bind where complementary probes are located + because of the label, the chip can be digitally analysed to determine an individuals base seq. for the gene of interest.
↳ digital analysis calc. strength of signal produced at each spot, w/ strength of signal representing level of gene expression in the individual
→ If the gene of interest is one which is known to affect the way the individual responds to a certain drug, then we now know their genotype with regards to this gene + medication can be perscribed accordingly.

› Gene expression → small variation to microarray technique
→ if gene is expressed, mRNA produced ∴ all mRNA extracted frome each tissue of interest (eg. normal + cancerous tissue)
→ R. transcriptase added to make cDNA, labelled, added to microarray + digitally analysed.
→ Researchers can see which genes are 'up-regulated' + 'down-regulated' in cancer cells, compared with normal cells.

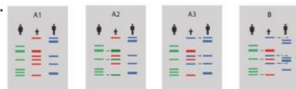
Genetic Fingerprinting

Analysing + comparing the DNA of individuals



1. Sample DNA, amplified by PCR, cut into smaller fragments using restriction enzymes
2. Fragments added to agarose gel + separated by size by electrophoresis
3. DNA in gel made single stranded (by heating) + copied onto a nylon mem.
4. Radioactive/fluorescently labelled DNA probes added to plate + bind via base pair complementarity.
   ↳ those that don't bind are washed off
5. Labelled DNA added to an x-ray film + appears as bands recognisable as a DNA fingerprint.

Note: thickness of bars indicates the no. of DNA fragments of a particular size
↳ distance fragment travels in a gel indicates its size



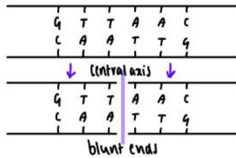
Uses: → crime scene analysis → immigration disputes → assess genetic variation in populations
→ paternity disputes → establish species membership

Restriction Endonucleases

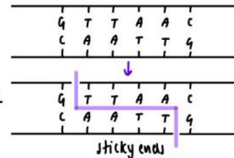
> Enzymes which cut the DNA at specific base sequences (→ recognition seq.)
↳ forms fragments

> Used by bacteria to defend against **bacteriophages**
↳ Cuts B.phage DNA into **smaller non-infectious fragments**
↳ By a **hydrolysis** reaction.

Blunt Ends
> Cut at positions directly opposite one another
> Difficult to join with other DNA.

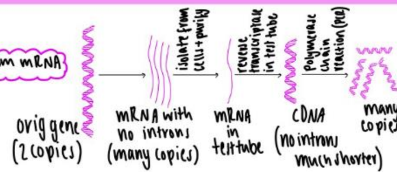


Sticky ends
> Cuts are staggered + produce single-stranded regions
> More useful as can form base pairs with other comp. sticky ends



Reverse Transcriptase

> Reverse transcriptase is an enzyme which catalyses the production of DNA from mRNA
↳ from retroviruses → copy their RNA into DNA in the host cell



How?
> **mRNA** for the required protein is isolated
> R. transcriptase used to obtain single stranded DNA using DNA nucleotides forming **complementary DNA (cDNA)**
> The mRNA strand is removed, + a 2nd strand of DNA is made by adding enzyme **DNA polymerase** + **more DNA nucleotides**
> Results in **double stranded DNA identical to the original DNA**
↳ produces the required gene needed to code for the protein

Inserting the Gene

> **Vector** = gene carrier, used to transfer DNA from one cell to another
> The gene is inserted into the vector + in a vector, it can be replicated + expressed

a.) Bacteriophages:
- **Viruses** which have a DNA core + protein coat
- **Infect bacteria** (host) by injecting its DNA into it
- Eg. human gene inserted into a herpes virus + transferred by infection into a human host cell where it is expressed
- If a bacteriophage has a DNA fragment spliced into its DNA it will transfer the recombinant DNA into the bacterial cell

b.) Bacterial plasmids:
- **Small, circular extrachromosomal DNA** (found outside the main loop of DNA), that occurs in certain bacteria (+ yeast)
- They replicate independantly + can be transferred from one bacterial cell to another
- Typically contain genes that provide **antibiotic resistance**.

Gene into vector
> Isolated gene + vector DNA cut using **same restriction endonuclease** to generate complementary sticky ends
> Mixed together + complementary sticky ends form **H bonds** + **DNA ligase anneals** fragments with **phosphodiester bonds**
> **Recombinant DNA**

* sticky ends might need to be added → complementary.

Vector into host cell
> Host cells freq. bacterial cells eg. Escherichia coli + Saccharomyces cerevisiae (unicellular + fast growing)

plasmid vector
> Recombinant plasmids mixed with bacterial cells in solution of **Ca^{2+} ions** + subjected to **heat shock** (rapid temp rise 0-40°C
> Causes cells to be **more permeable**, encouraging the bacterial cells to take up the plasmid.

B.phage vector
> Recombinant viruses mixed with bacterial cells
> Viruses infect some of the bacterial cells, **firing** the **R.DNA** into the bacterial cells.

Identification of Transformed Cells

1. Using antibiotic resistant genes

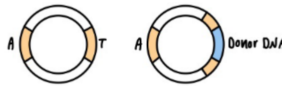
- Plasmids almost always contain antibiotic resistant genes
  eg. **R-plasmids** resistance genes for **tetracycline + ampicillin**.
- Restriction endonuclease cuts middle of tetracycline
  - **Fail** to take up plasmids : killed by both A and T
  - Take up **origin** plasmids: not killed by A or T
  - Take up **recombinant** plasmids: resistant to A, killed by T
- Bacteria cultured on **agar plates**, multiplies to form **colonies**
- **Replica-plated** ('blotting' the original plate with a pad + then transferred) + treated with antiBs. pressing it on the surface of a fresh plate so that a few cells from each colony are

2. Fluorescent marker genes

- **Fluorescent protein gene** added to a plasmid
- Donor DNA added in middle of fluorescent gene
- Transformed cells will not be able to fluoresce.

3. DNA probes

- Can be used to identify if host DNA is recombinant



Selection + Cloning of Transformed Cells

↳ **GMOs/GEMs**

- Transformed bacteria are **cultured**, allowing their **DNA to replicate**, b4 the cells divide by mitosis → many clones of the desired gene are produced.
- **Clone**: a group of cells/organisms/genes that are exact copies of each other.
- Grow + divide rapidly, **synthesises** large amounts of the protein coded for on the gene, **protein extracted + purified**
- Genes can be cloned by growing bacteria in **industrial fermenters**.

Advantage: Replicate rapidly. Only when a certain cell density is reached are the genes responsible for making the required product switched on. In this way, much larger amounts of product can be produced much more rapidly.

Disadvantage: A problem with large industrial-scale fermenters is that respiring micro-organisms produce a lot of heat. Cooling a fermenter is expensive so genetic engineers are experimenting with the use of thermophilic bacteria (normally live in places where temp is high eg. hot springs).

Therapeutic Genetic Modification

GMO: genetically modified organism
GEM: genetically engineered microorganism

1. Use of GM viruses to treat cancer:

- 2015, study published had used **genetically modified herpes** (cold sore) virus to treat **malignant melanoma**.

a.) could **no longer make a protein** which normally enables the virus to **reproduce** inside healthy cells. Hence, unable to cause cold sores. However, since cancer cells themselves produce this protein, the virus was **only able to reproduce inside cancer cells**.

b.) viral gene which normally **inhibits antigen-presentation on virus-infected cells** was disrupted, so that the infected cells become **more 'visible'** to the **immune system**.

c.) **human cytokine gene** inserted which **triggers an immune response** to the infected cells.

- Trial had some success + research continues into viral therapies for other cancers inc. bowel + prostate cancers.

2. Use of GM viruses to treat bacterial infections:

- Viruses GM to improve **efficacy of antiB.** against bacterial infections
- Some antibiotics target bacterial DNA, but bacteria have a **defence mech.** which helps them **repair DNA** targeted by these antiB.
- The GM viruses **disrupt this DNA repair action**, allowing the antiB. to work
- In 1 study, mice with both GM virus + antiB had an **80%** survival rate compared with a **20%** for those with antiB alone.

3. Use of GM bacteria to produce a wide range of substances

- Insulin
- Interferon
- Enzymes
- Adhesives
- Lung surfactant protein
- Human growth hormone

Transgenic Organisms

Transgenesis: the transfer of genes from one organism to another.
Transgenic organism: an organism that develops from a cell into which donor DNA (transgene) has been introduced

- Allows traits to be introduced that are not present in an organism naturally
- The recombinant DNA integrates into one of the organism's chromosomes (at the one cell fertilised egg stage) + is passed on from gen. to gen. with the organisms DNA.

Animals:
- → encourage faster growth rate + better food quality traits
- → produce substances of medical + pharmaceutical value
  - ↳ transgenic animals (eg. sheep, cattle, pigs, rabbits, chickens) instead of fermenters
  - ↳ more cost effective as expensive culture-vessels for large-scale production, continuous monitoring of equipment + maintenance aren't required.
  - ↳ animals such as sheep + cattle can add sugar residues to the proteins which makes them more effective + versatile synthesisers of desirable products than most microorganisms
  - ↳ future demand for a product can be met by interbreeding transformed animals.
  - ↳ Interferon, blood clotting factors VIII, alpha-1-antitrypsin, human serum albumin, haemoglobin, vaccines
- → use as models in human disease research

- **Liposomes** - encapsulate the donor DNA in lipid vesicles, can cross the lipid bilayer of cell mem.
- **Electroporation** - makes the cell mem. permeable by disrupting it with high voltage treatment
- **Viruses**
- **Fertilised eggs** - inserting donor DNA directly will ensure the animal will contain recombinant DNA.

Plants:
- → produce higher crop yields, increased variety, better food quality traits (↱ help with global food production)
  - ↳ increased shelf-life by controlled ripening, nutritional enhancement
- → produce pest + disease resistant crops
- → cultivate GM crops that grow in unfavourable environments

- **Soil bacterium *Agrobacterium tumefaciens*** causes tumour like plant galls as integrates bacterial DNA in the form of a plasmid into DNA of plant
  - ↳ genetic engineering removes gall forming aspect + replaced with donor DNA that will be integrated into the host.
  - ↳ limited due to host specificity.
- **Gene guns** - coating microscopic pellets (tungsten/gold) with donor DNA + firing into the host cell
  - ↳ less reliable but works with species resistant to the agrobacterium

Ethical issues: not always based on scientific evidence
- Not natural - but same principle to selective breeding
- Super weeds
- Greater ecological range resulting in them outcompeting non crop species
- Insects becoming resistant to the toxic effects of GM crops
- Allergies caused by compounds from GM crops

Gene Therapy

Inserting 'normal gene' (functional allele) into an organism's body to restore metabolism + eliminate disease caused by a genetic disorder due to absent or faulty genes

Somatic-cell gene Therapy:

- Targets affected tissue only
- accessible parts of the body eg. lungs
- used at any stage of life
- Blood, liver, skin, bone marrow cells

- Extracting cells → biopsy syringe under local anaesthetic
- Genes into cells → retrovirus, adenovirus (respiratory), liposome
- Donor DNA can be incorporated into the host DNA or function independantly (episomes) in the nucleus

Germ-line gene Therapy:

- Targets fertilised egg to replace the defective gene
- Raises many moral + ethical issues that its approach is not permitted

Problems:

- Insertion of new gene may disrupt another normal host gene
- The virus may cause toxic side effects in the patient
- Short-lived nature of gene therapy
- Immune response
- Multigene disorders

Advantages:

- Giving someone born with a genetic disease or cancer the chance at a normal life

Ethical issues:

- What is normal + what is a disability or disorder, + who decides?
- Are disabilities diseases? Do they need to be cured or prevented?
- Does searching for a cure demean the lives of individuals presently affected by disabilities?
- Somatic gene therapy more/less ethical than germline?
- V. expensive so who will have access + who will pay for their use?

Haemophilia B

- successfully treat haemophilia B. (sex-linked blood clotting disorder, caused by a recessive allele found on the X-chromosome)
  - ↳ usual treatment involves injection with Factor IX, the clotting factor not produced in sufferers.
  - ↳ In gene therapy trial, patients injected with viruses vectors for a healthy copy of the gene for Factor IX.
  - ↳ viral vector delivered healthy gene to cells in the liver, where gene successfully directed synthesis of Factor IX.
- Success to learning from the problems encountered in other trials:
  - ↳ virus used as a vector did not normally infect humans + ∴ presumed that none of the patients would already have immunity to it.
  - ↳ steroids given to suppress immune responses to the injected virus.
  - ↳ virus selected since rarely inserts itself into hosts chromosomes, ∴ reducing chances of serious side effects eg. cancer, which have occurred in other gene therapy trials.

Cystic Fibrosis

- Gene therapy less successful for cystic fibrosis → lack of gene which produces a transport protein
  - ↳ target cells in the lungs are regularly renewed + so repeated doses of gene therapy need to be given.
  - ↳ viral vectors used in the past have induced immune responses.
  - ↳ Recent studies have had modest success with a monthly treatment involving liposome vectors + it is thought in 5-10 yrs, gene therapy will at least be an effective treatment for CF, if not an outright cure.
- liposomes → sprayed through nose into lungs + taken up by lung cells
  - ↳ problems: fusion to lungs not always successful, temporary, some toxic to some cells
  - ↳ advantage: protect the DNA + can carry large pieces of DNA
- Adenoviruses → sprayed into nasal passages
  - ↳ problems: toxic side effects, can cause serious lung infections, some individuals may be immune to them

Gene Sequencing

Genome: complete DNA seq. one one set of chromosomes in diploid, eukaryotic organisms
Genome seq: determining the order of nucleotides + so the genetic code

	HGP begins (1990)	HGP completed (2003)	2015
Time required to generate a human genome	6-8 years	3-4 months	<1 day*
Cost of generating a human genome	$1 billion	$10-50 million	$1000*

- This info is v. useful in a no. of areas, including determining causes of disease + tailoring treatment, investigating evolutionary relationships, genetic testing + forensics
- Microarray still used due to associated costs of gene seq. but becoming more rapid + less expensive
- Knowledge of seq. of bases in a particular gene enables the 1° structure of protein encoded by that gene to be worked out
  ↳ molecular modelling software predict 2°, 3° + 4° structures

- 1st genomes 2 b fully seq. were of viruses (1970/80s).
- By 2000, 1st bacterial (haemophilus influenzae), fungal (saccharomyces cerevisiae), plant (Arabidopsis thaliana) + animal (caenorhabditis elegans) sequenced genomes had been reached.
- Knowledge of genetic code grew as more species genomes were seq.
- Many previously held theories about evolutionary relationships + taxonomy have been revised in light of genome seq. info.

- DNA is a persistant mol. + scientists have been able to successfully extract it even from organisms which have been dead for many years.
- In 2010, 1st draft of genome seq. for Neanderthals produced using DNA from fossilised remains of individuals who lived in Europe 40,000 yrs ago.
- Species extinction thought ~30,000 yrs ago + shares a common ancestor with Homo sapiens
- Genome seq. project has contributed to knowledge of human evolution + migration patterns

Human Genome Project (1990-2003)

- 21000 protein coding genes → exons = 1-2% of total genome.
- ~6000 RNA genes → genes which are transcribed into RNA but not subsequently translated
  → eg tRNA, rRNA + many others with unknown functions (some have been implicated in certain types of cancer + in Alzheimers)
- 'Junk' DNA → range of characteristics eg large proportions make up gene introns, structural DNA (heterochromatin) + sections called 'transposable elements' which can move around in the genome

Social + Ethical implications of gene sequencing:

- Direct-to-consumer gene sequencing products eg 23andme kit, has important social implications
- Enables to see if carry genes which may increase their risk of developing diseases later in life + how metabolise various common medications.
- No guidance if an allele tested is positive
- Concerns over who else might request access to the info eg. doctors, family, insurance providers + potential employers

Knockout + Knockin Mouse

Knockin: particular gene added → used to add defective genes to study the disease progression
Knockout: genetically engineered (transgenic) organism that carries a gene which has been made inoperative → disabled, removed, replaced with defective alleles
↳ provides info about the gene by comparing how the knockout organism differs from not modified individuals
↳ gene's role in protein production + its influence metabolically + physiologically

Mouse: model organism for the study of genes
↳ inactivation/replacement can be used to study the dev. of genetic disorders + drug therapies

Pharmacogenetics

> The tailoring of drug treatments to individuals based on their genotype

- Microarrays + gene sequencing have made major contributions to advances in this field, by **providing info. on an individuals genetic makeup.**
- Due to genetic variation, people can **respon differently to certain drugs** → **ineffective / dangerous**
  → **dosage may vary**
- Different **rates of metabolising painkiller codeine** (poor, intermediate, extensive, ultrarapid) depending on which alleles they possess
  - ↳ **diff alleles = diff. levels of activity of an enzyme** involved in the conversion of codeine to morphine. (high levels of morphine v. dangerous).
    - ↳ cytochrome P450 enzymes influence metabolism of many other drugs aswell.
  - ↳ **effective dosage diff. for each group**. Ultrarapid → toxic effects, life threatening. Poor → completely ineffective.
  - ↳ Some ethnic groups eg. Africans + African-Americans, increased incidence of ultrarapid g.type. → alleles involved determined by microarray

- Personalised medicine explores how treatment can be personalised to improve clinical outcome
- Designer drugs can be matched to an individuals genetic profile
- Dev. of 'designer drugs' will make **adverse drug reactions + side effects less common** + should ensure that patients are perscribed the **right treatment** + **dosage 1st time round**.

Ethical Issues

	Potential Benefits	Potential Risks/Concerns
GEMs developed to produce protein	more economic + wider production of medically important proteins eg. insulin	Some of the micro.o (eg E.coli) live normally in the human gut; GEMs could escape from lab + create a new strain of 'superbug'
GM plants inc. GM crops	Cheaper food for richer countries, possible reduction in use of pesticides, reduction of food shortages in poorer countries.	Risk of 'genetic pollution' with the spread of new genes from the mod. crop to wild species → super weeds; ecological concern that GM plants may out-compete wild plants; allergic reactions following consumption of GM foods
GM animals for food.	Increased productivity of animals eg fish + cattle by transferring growth hormone gene into their genome	Concern that foreign protein, produced by transferred genes, may act as antigens (allergens) + increase likelihood of allergies
Gene therapy	Effective treatment of genetic diseases eg CF, relieving suffering + increasing life expectancy.	Introducing genes into human genome may disrupt the functioning of other genes, as in the appearance of leukaemia in patients treated for severe combined immunodeficiency (SCID)
Human genome research	Facilitates biomedical research	Concern than info from genome research might be used to produce 'designer babies' (eg. looks + high IQ); individual's genomic info (eg. susceptibility to heart disease) available to insurance companies
Genetic screening	Better understanding of risk of passing on a genetic disorder; foetus may be tested for the disorder b4 birth (prenatal diagnostic testing)	Increased risk of stress resulting from the knowledge of being a carrier or of developing a disorder later in life (eg. Huntington's); termination of pregnancy may not be acceptable
Gene knockout tech	Better understanding of how genes function; these genes might be implicated in a genetic disorder or might mutate to cause cancer	Large no. of mice used in biomed. research, many of which may be in pain; view that animals have rights + that it is unacceptable to use them in this way.

Safety Techniques

Containment measures:

- Purpose built labs - air filters
- Controls for access + cleaning
- Disinfectant procedures
- Bacterial strains ill-adapted to human physiology (optimum temp. diff. to human)
- Strains with 'suicide genes' that get activated if specific temp or pH changes

Legislation

With so many views there is need for gov. to make decisions + ensure legislation is in place