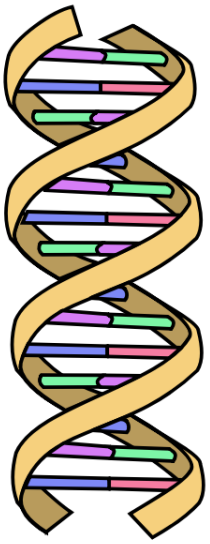
DNA & Division

Structure of DNA

- Structure of DNA is composed of:
  - Two complementary, long anti-parallel (5' to 3' and 3' to 5') strands.
  - Bonded together by hydrogen bonds between nitrogenous bases in between the strands.



= Adenine

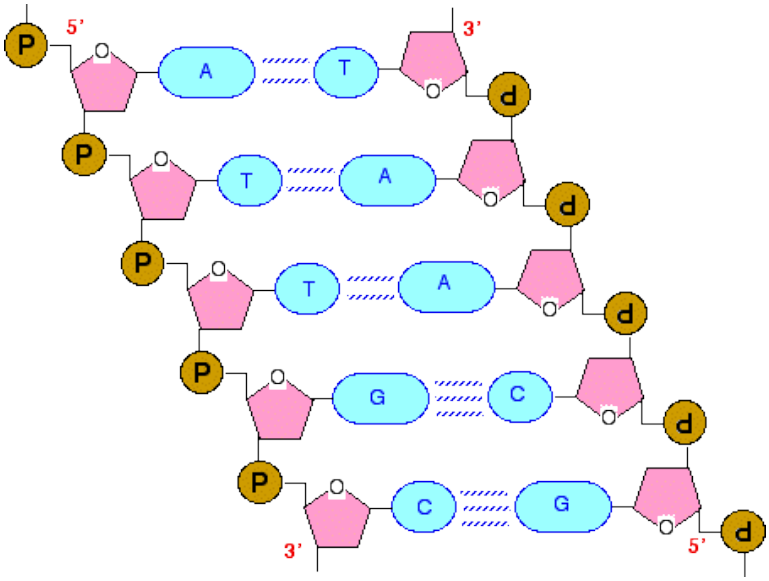
= Thymine

= Cytosine

= Guanine

= Phosphate backbone

DNA



Structure of RNA

- The structure of RNA is composed of:
  - A single stranded molecule with:
    - Ribose sugars
    - Phosphates
    - Nitrogenous bases (Uracil, Guanine, Adenine, and Cytosine)

Differences	
DNA	**RNA**
Found in Nucleus	Found in Cytoplasm
Double Stranded	Single stranded
Long	Short
Bases are: G, C, A, T	Bases are: G, C, A, U
Deoxyribose sugars	Ribose Sugar
Encodes inheritance information	Transcribing & Translation

DNA Replication

1. DNA strands are unwinded and unzipped by DNA Helicase. A replication fork is formed.
2. RNA primers start the first few sequences of a DNA strand. Performed by primase.
3. DNA polymerase finishes RNA primer strands, using template strands. Performed in a 5' to 3' direction.
4. Primase builds RNA primers on the lagging strand, for DNA polymerase to later continue building on. The primers/pieces are called Owazaki Fragments.
5. Exonuclease removes primers, and DNA polymerase fills the gaps.
6. DNA Ligase connects fragments together by phosphodiester bonds.

Compare	**Contrast**	
Double Stranded	**Eukaryotic Chromosomes**	**Prokaryotic Chromosomes**
	Coiled around histones.	No protein complex.
	Found in nucleus.	Found in cytoplasm/nucleoid.
	Linear chromosomes.	Circular chromosomes.
	Multiple chromosomes.	Singular chromosomes.

Binary Fission

1. DNA replicates.
2. Cell elongates, and DNA is separated.
3. Cross wall forms.
4. Cross wall forms completely.
5. Two daughter cells are formed.

Binary Fission vs Mitosis	
Binary Fission	**Mitosis**
Used for asexual reproduction.	Used for growth, replacement, and repair.
Only for prokaryotes.	Only for eukaryotes.
No spindle fibres used.	Spindle fibres are used.
Only replicates one chromosome.	Replicates multiple chromosomes.

How Cytokinesis/Nuclear division occurs

1. Microtubule filaments form a concentric ring around the equator of the cell.
2. The microfilaments contract to form a cleavage furrow, which pulls the membrane inwards.
3. When the furrow meets in the centre, the cells completely pinch off and form two daughter cells.

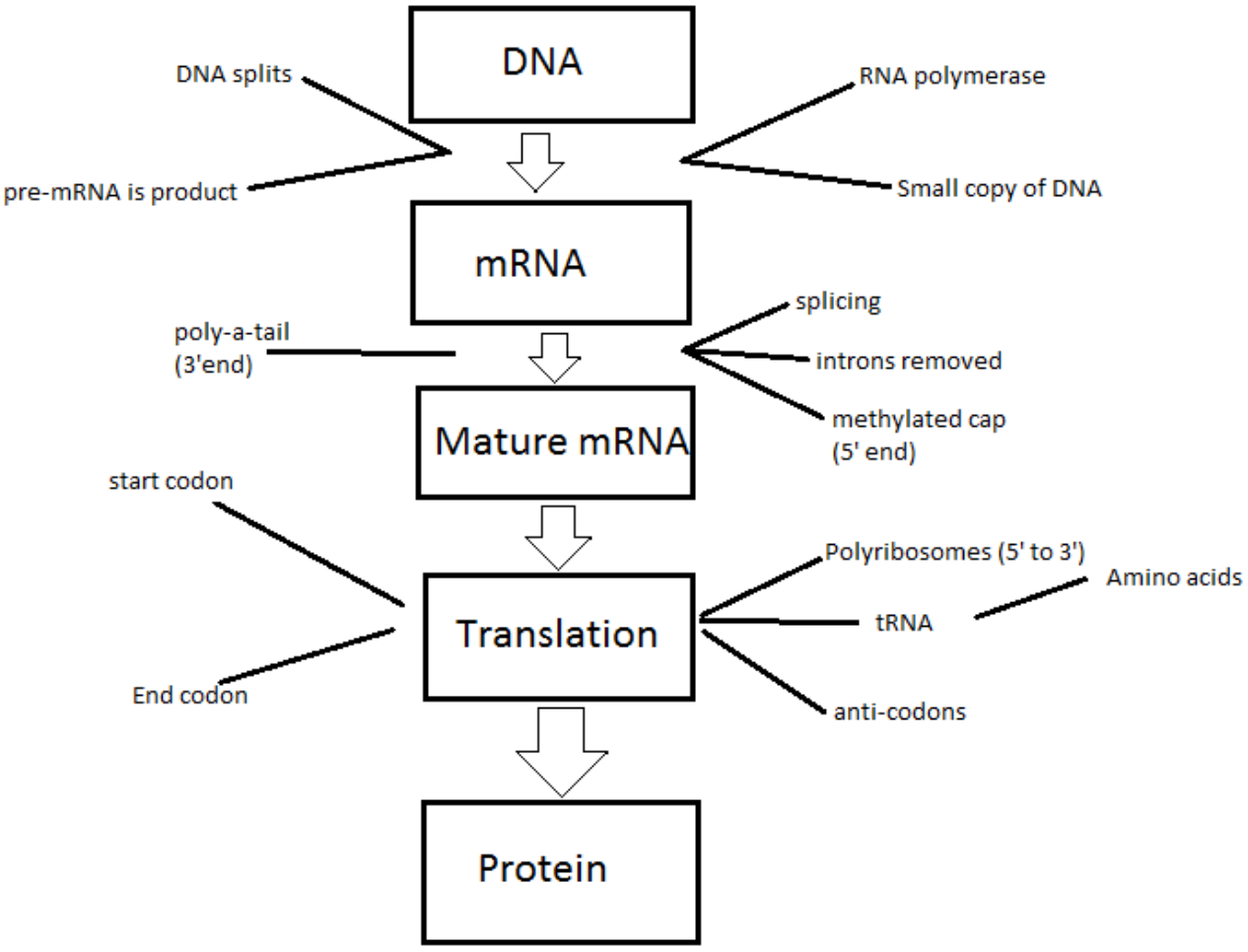
Mitosis vs Meiosis	
Mitosis	**Meiosis**
Used for growth, repair, and replacement.	Used for sexual reproduction.
Occurs in somatic cells	Occurs in gametes.
Has 1 cell division.	Has 2 cell divisions.
Parent cell is diploid (2n, 46)	Parent cell is diploid (2n, 46)
Daughter cell is diploid (2n, 46)	Daughter cell is haploid (n, 23)
Produces 2 daughter cells.	Produces 4 daughter cells.
Asexually reproducing organisms use Mitosis to reproduce.	Sexually reproducing organisms use Meiosis to reproduce.
Variation is not produced, except for when mutations occur.	Variation is produced by crossing over, and independent assortment.

<u>Apoptosis – Programmed Cell Death</u>

- Apoptosis occurs when:
  - A cell reaches end of its lifespan.
  - A cell becomes infected.
  - A cell becomes damaged.
- Apoptosis is important because:
  - It maintains cell numbers.
  - Defends against damaged/dangerous cells.
  - Shapes embryonic tissue → hands and toes aren't webbed.

1. Cell shrinks + loses volume.
2. Nuclear membrane degrades, and plasma membrane forms blebs.
3. Nucleus collapses into small spheres and DNA breaks apart.
4. Cell breaks into apoptotic bodies to be engulfed by macrophages.

Genetic Code



- Phenotype – The physical trait/characteristic created by a certain gene.
- The role of an enzyme is to help with bodily reactions such as digestion, neuromuscular junctions, and cellular respiration.
- The genetic code is the sequence of nucleotides that determines the specific amino acid sequences in protein synthesis.
- DNA codon → mRNA codon → amino acid → protein

Transcription

1. **(Initiation)**
   RNA polymerase binds to DNA at a promoter sequence, which signals the start of a gene.

2. **(Elongation)**
   RNA polymerase unwinds DNA and begins adding complimentary RNA nucleotides to the template strand, in a 3' to 5' direction.

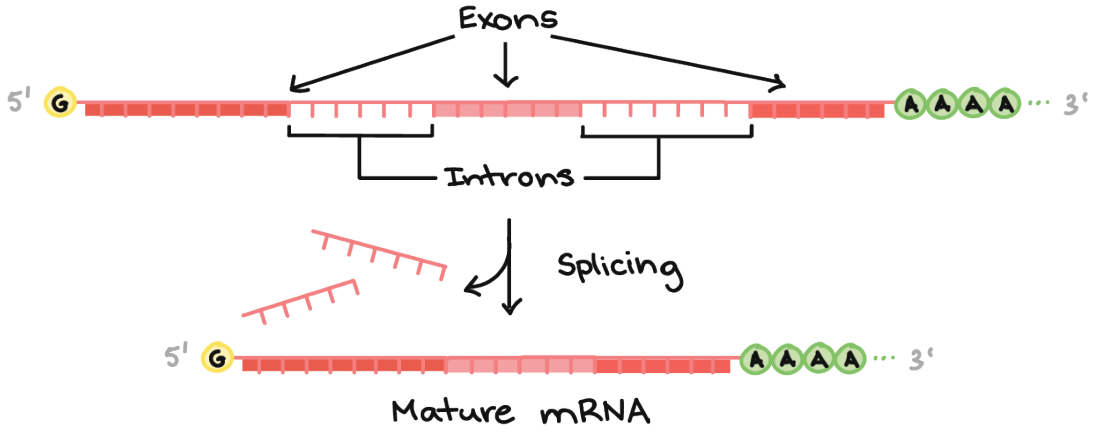
3. **(Termination)**
   RNA polymerase continues to elongate until it reaches a terminator sequence, which releases the pre-mRNA
   .
4. DNA winds back up into a DNA helix.

Pre-mRNA processing

1. **(Capping)**
   A methylated cop (guanine nucleotide) is added to the 5' end of the pre-mRNA to protect it from enzymes.

2. **(Polyadenylation)**
   A poly-A-tail (100-200 adenine nucleotides) is added to the 3' end of pre-mRNA to allow it to 'swim' and protects it from enzymes.

3. **(Splicing)**
   Introns (unwanted sequences) are removed and the remaining exons are connected to form a single molecule of mature mRNA.



Pre-mRNA vs mature mRNA	
Pre-mRNA	**Mature mRNA**
Introns are present in structure.	Exons are only present in structure.
No poly-a-tail.	Poly-a-tail is present on 3' end.
No methylated cap.	Methylated cap is present on 5' end.

Translation

1. **(Initiation)**
   mRNA moves through the nuclear pore into the cytoplasm. A small ribosomal subunit attaches itself to the 5' end of the mRNA strand.

2. **(Initiation)**
   The small subunit scans mRNA for an AUG start codon, in a 5' to 3' direction. Once it reaches the start codon, a large ribosomal subunit attaches and a tRNA molecule with an anti-codon matching the start codon binds to the start codon.

3. **(Elongation)**
   The ribosome moves to the next codon, in a 5' to 3' direction, and another tRNA molecule with the complimentary anti-codon joins onto the ribosome, adding another amino acid into the protein chain.

4. **(Elongation)**
   As the ribosome moves along the mRNA strand, tRNA, peptide bonds form between the amino acids, adds more amino acids and a protein chain is formed.

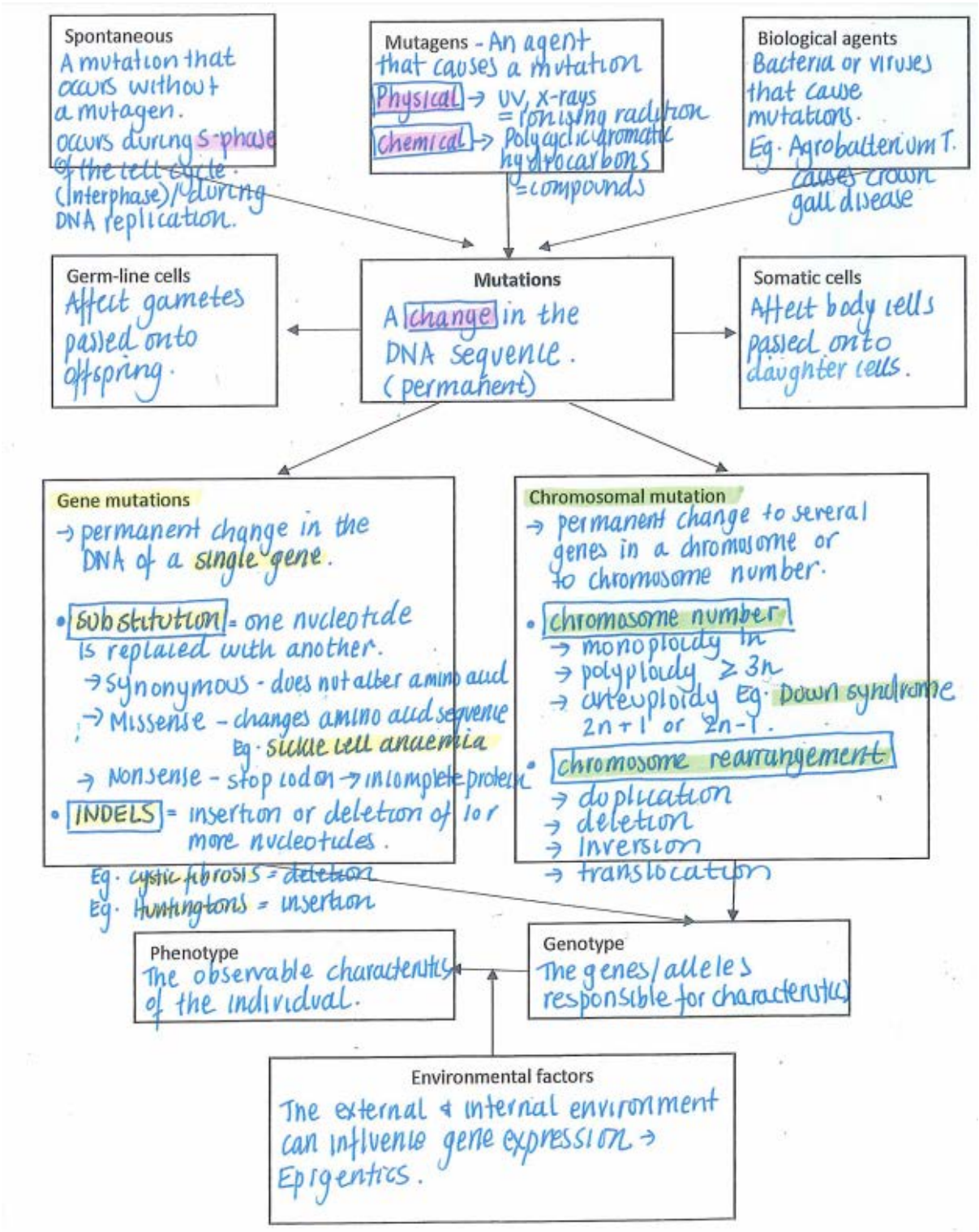
5. **(Elongation)**
   As this process continues the protein is elongated.

6. **(Termination)**
   Once the ribosome reaches the stop codon on the mRNA molecule, translation ends and the protein is released.

Gene Expression/Suppression

- **Nucleosome –** A 'basic unit' of DNA packaging.
  - **Consists of DNA wrapped twice around eight histone protein cores.**
- **Gene Expression** – The expression of Genes. Is affected by:
  - Demethylation of DNA
  - Deacetylation of histones.
- **DNA Methylation –** Occurs when a methyl group is added to a cytosine base on DNA.
  - Methyl group increases the negative charge of DNA, making the nucleosomes tightly coiled.
  - Suppresses genes.
- **Histone Acetylation –**
  - Acetyl groups attaches to histone proteins.
  - Acetyl groups 'weaken' the charges of histones, making nucleosomes loosely coiled.
  - Expressions genes.

Genetics


Spontaneous
A mutation that occurs without a mutagen.
occurs during S-phase of the cell cycle (Interphase) during DNA replication.
Mutagens - An agent that causes a mutation
Physical → UV, X-rays = ionising radiation
Chemical → Polycyclic aromatic hydrocarbons = compounds
Biological agents
Bacteria or viruses that cause mutations.
Eg. Agrobatterium T. causes crown gall disease
Germ-line cells
Affect gametes passed onto offspring.
Mutations
A change in the DNA sequence. (permanent)
Somatic cells
Affect body cells passed onto daughter cells.
Gene mutations
→ permanent change in the DNA of a single gene.
• substitution = one nucleotide is replaced with another.
→ synonymous - does not alter amino acid
→ Missense - changes amino acid sequence
Eg. sickle cell anaemia
→ Nonsense - stop codon → incomplete protein
• INDELS = insertion or deletion of 1 or more nucleotides.
Eg. cystic fibrosis = deletion
Eg. Huntingtons = insertion
Chromosomal mutation
→ permanent change to several genes in a chromosome or to chromosome number.
• chromosome number
→ monoploidy n
→ polyploidy ≥ 3n
→ aneuploidy Eg. Down syndrome 2n+1 or 2n-1.
• chromosome rearrangement
→ duplication
→ deletion
→ inversion
→ translocation
Phenotype
The observable characteristics of the individual.
Genotype
The genes/alleles responsible for characteristics
Environmental factors
The external & internal environment can influence gene expression → Epigentics.

Mendel's Laws

- Law of Dominance
  - There are two alleles for each gene.
  - One is recessive.
  - One is dominant.
- Law of Segregation
  - Two alleles segregate into separate gametes, and re-join at fertilisation.
- Law of Independent Assortment
  - Pairs of alleles for one gene segregate independently of other genes.

Test Cross

- Used to determine if an organism is homozygous dominant or heterozygous.
- The organism is crossed with a homozygous recessive organism.
  - If the organism is heterozygous:
    - 50% of offspring express dominant phenotypes.
    - 50% of offspring express recessive phenotypes.
  - If the organism is homozygous:
    - 100% of offspring express dominant phenotypes.

Blood Type	Genotype
O	ii
AB	$I^A I^B$
A	$I^A I^A \ or \ I^A i$
B	$I^B I^B \ or \ I^B i$

Polygenetic Inheritance

- Polygene
  - A gene that has multiple alleles contributing towards it.

Continuous Variation vs Discontinuous Variation	
Continuous Variation	**Discontinuous Variation**
Polygenic.	Monogenic.
Shows a range of phenotypes.	Shows a limited number of phenotype.
Genes have an additive effect.	-
Strongly influenced by environment.	Not influenced by environment.

Biotechnology & Genetic Techniques

- Genetically Modified Organism (GMO)
    - An organism whose genome has been affected by genetic engineering.
    - E.g. Roundup ready canola and Bt cotton.
- Transgenic Organism
    - An organism that has foreign DNA in its genome.
- Restriction Enzymes
    - Enzymes that cut DNA at recognition sites to make a desired gene cut out.
    - Found in bacterial cells, as a self-defence mechanism.
    - Sticky ends on a restriction fragment has exposed nucleotides, which makes it easier to recombine because of adjacent nucleotide pairing.
    - Blunt ends have no exposed nucleotides, making it harder to recombine.
- Recombination
    - DNA Ligase is used in recombination.
    - It joins a restriction fragment to a host's DNA, via phosphodiester bonds.
    - Chances of successful recombination occur by:
        - Using the same restriction enzyme on the host DNA and restriction fragment.
        - Making sticky fragments.

<u>Polymerase Chain Reaction (PCR)</u>

1. **Denaturation – 95 degrees Celsius**
    - DNA sample heated to 95 degrees.
    - Hydrogen bonds between nitrogenous bases break down.
    - DNA strands separate via denaturation.
2. **Annealing – 50-60 degrees Celsius**
    - DNA sample is cooled to 50-60 degrees.
    - Primers are added to the mixture.
    - Primers join onto complimentary sequences.
3. **Extension – 72 degrees Celsius**
    - Mixture is heated to 72 degrees Celsius, the optimal temperature for Taq polymerase.
    - Taq polymerase adds nucleotides to primers, causing extension of primer strands.

<u>DNA Visualisation</u>

- DNA can be visualised with three methods:
    - Gel electrophoresis.
    - DNA probes & Microarray technology.
    - DNA sequencing.

Gel Electrophoresis

- Components needed:
    - Agarose Gel
    - Electrophoresis chamber.
    - Marker sample.
    - Dye.
    - Buffer solution.
    - DNA sample.
    - Electric current.

1. Agarose gel is placed in electrophoresis chamber and is filled with buffer solution.
2. Positive and negative electrode is attached to each end of the chamber.
3. DNA sample and dye is pipetted into the wells.
4. Electric current is applied.
5. Post electrophoresis stain such as ethidium bromide is added, which binds to DNA and is fluorescent under UV light.
6. Size and molecular weight of each fragment is determined.

DNA Profiling

- DNA profiling is used to identify an individual from a sample of DNA.
- Done by analysing patterns in introns of DNA.
    - Introns mainly have two types of repeated sequences:
        - STR's (Short Tandem Repeats) – 2-5 bp.
        - VNTR's (Variable Nucleotide Tandem Repeats) - >5 bp.
- Short Tandem Repeats (STR's)
    - Regions of non-coding DNA that contains repeats of the same nucleotide sequence.
    - Highly variable among individuals.
    - Effective for identification.

1. DNA is extracted from DNA sample.
2. DNA sample is amplified using PCR, and mixed with primers that attach to STR sites.
3. Gel electrophoresis is conducted.
4. Data is visually analysed.

Sanger Method

1. Region of DNA to be sequence is amplified by PCR & denatured to produce a single strand.
2. A mixture containing **DNA, primer, DNA polymerase, and four deoxynucleotides (dA, dC, dG, dT)** is prepared.
3. Equal amounts of the mixture is placed into four test tubes.
4. A dideoxynucleotide (ddA, ddC, ddG, ddT) is added to terminate synthesis of DNA strands.
5. Contents of tubes are transferred to four wells on electrophoresis gel. Electrophoresis is conducted.

Read from downwards to upwards.

Microarray Technology

- A glass slide that consists of thousands of DNA probes arranged in an array.
- Used to determine gene expression, diagnosing diseases, or comparing gene expressions.

1. A microarray is prepared, which consisted of thousands of probes fixed to glass/silicon in an array.
2. mRNA is isolated from tissue sample.
3. Reverse transcriptase is used to make stable complementary DNA (cDNA), using fluorescent nucleotides.
4. Mixture is applied to microarray.
5. Any cDNA complementary to a sequence on the array will bind to it → Nucleic acid hybridisation.
6. Microarray is scanned for presence of fluorescent dye (from cDNA). This detects the genes present and determines gene expression.

- Gene probe
    - A specific short length of single stranded DNA with a fluorescent tag attached.
    - Complementary to known DNA sequences.
    - When mixed with a sample of DNA, the probe binds to the complementary sequence, which will show up in UV light.

Gene Cloning

1. Plasmids are extracted from bacteria.
2. A restriction enzyme is used to cut the plasmid DNA and desired gene's DNA.
3. DNA ligase binds desired gene and plasmid together, and a recombinant plasmid is produced.
4. Recombinant plasmids are added to a bacteria culture. Bacteria take up plasmids, replicate, and multiple copies of the desired gene is made.

Biotechnology in Agriculture

BT Cotton	• Cotton modified to produce a pesticide. • BT bacterium contains a gene that produces proteins toxic to caterpillars. • BT bacterium gene is transferred to cotton plants.
Roundup Ready Canola	• Plants exposed to glyphosate are unable to produce amino acids, and die. • Roundup ready canola has two genes inserted into its genome via bacteria, which helps it withstand glyphosate herbicide.
Golden Rice	• GMO rice plants that produce beta-carotene, which is required to produce vitamin A. • Improves public health.
GM Salmon	• Salmon engineered to have faster growth rates. • Genes for growth-rate hormones from other species are inserted into salmon genome.

Arguments For and Against GMO Crops.	
For GMO	**Against GMO**
Biotechnology is natural. It is an extension of selective and crossbreeding.	Biotechnology is not natural. It involves transferring genes between species, which happens rarely in nature.
Decreases the use of pesticides.	Encourages uses of pesticides and herbicides, damaging the environment.
Can improve public health.	-
Can combat global food crisis. (Increased growth rates, capacity, and yield)	-
Little transfer of genes between species.	Gene transfer between species can make super weeds/pests, which leads to lesser genetic diversity.
N	Long term effects of GMO consumption is unknown.

Biotechnology in Humans

Genetic Testing	Analysis of an individual's DNA, chromosomes, or products of genes. Can confirm or rule out suspected genetic disorders, or determine chances of it being passed on. Used for pregnancy screening.
Production of pharmaceuticals using gene cloning	Functional genes are inserted into bacteria using a plasmid vector. Allows for the production of large amounts of a desired protein. E.g. Human Insulin and Human Clotting Factor VIII.
Gene Therapy	Normal and functional genes are delivered to individuals to compensate for disease causing mutations. Somatic Cell Therapy (Body cells – no inheritance to offspring) Germ Line Cell Therapy (Sex cells – Inheritance to offspring – Controversial as mistakes can be passed on)

Nuclear Transfer Cloning

1. DNA is removed from diploid nucleus of a somatic cell from Organism A.
2. Unfertilised egg is removed from Organism B.
3. Nucleus/DNA is removed from egg.
4. DNA from organism A is inserted into egg.
5. Fused, fertilised cell develops into an embryo in a foster mother.
6. Organism that is born is identical clone of Organism A.

Stem Cells

Type of Stem Cell	Source of Stem Cell	Potency of Stem Cell
Embryonic Stem Cells	Inner cell mass of Blastocyst	Pluripotent Can differentiate into any cell in the body, except cells that make up embryonic membranes.
Adult Stem Cells	Adult Tissue	Can specialise into a limited range of cells.

Evidence of Change

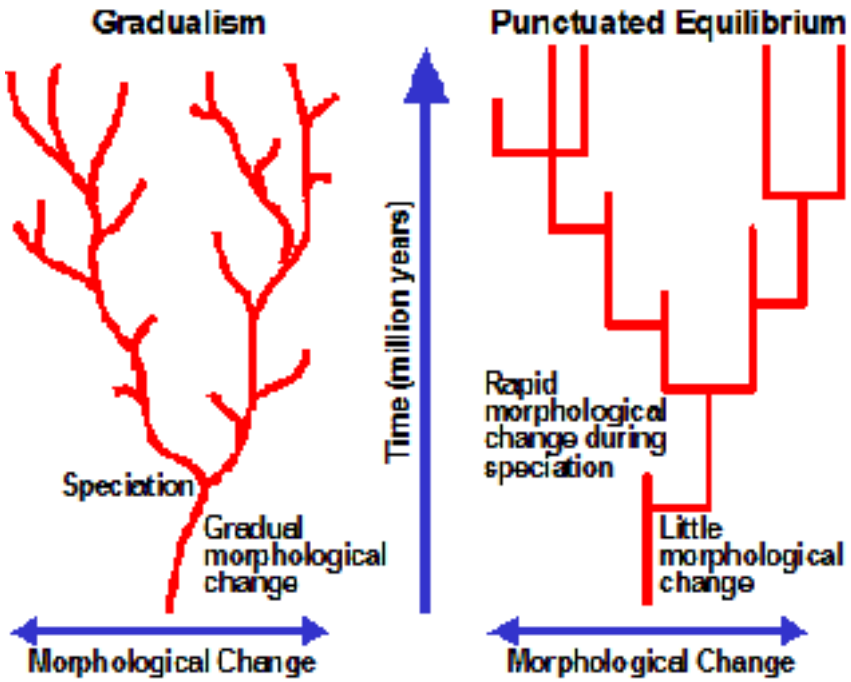
- Evolution
    - The gradual change in the gene pool of a population of organisms, that results in the formation of a new species.

Evidence for Evolution

- **Fossils**
    - The remains of a prehistoric organism that has been embedded into rock and preserved in a petrified state.
    - Can show the exact lengths of an organism from prehistoric eras.
    - Fossils can show transitional organisms.
        - E.g. Archaeopteryx, the transition organism from dinosaur's → birds.
- **Comparative Anatomy**
    - **Homologous Structures**
        - Structures that are the same between organisms but have different functions.
        - E.g., pentadactyl limb shared between arms of a human, wing of a bat, flipper of a whale, leg of a dog.
    - **Vestigial Structures**
        - Structures that are present in organisms that have no function, but are very vital in function for ancestral organisms.
        - E.g. Appendix in humans.
    - **Analogous Structures**
        - Structures that are different between organisms but have the same function.
        - E.g. wings of a bat and insect.
- **Comparative Embryology**
    - The comparison of embryonic development between species.
- **DNA and Protein Similarities**
    - The comparison of DNA/Amino acid sequences for certain cells/chemicals between species.
    - E.g. Amino acid sequence of haemoglobin between humans and other species.

Important Dates

- The first life forms appeared 3.8 billion years ago.
- The first invertebrates appeared in the Cambrian Era, 570 mya.
- The first reptiles appeared in the Permian era.
- Eurasia and Gandwana unite to form Pangea in the Permian Era.
- A mass extinction event occurs in the Triassic Era.
- The Archaeopteryx appears in the Jurassic Era.
- Pangea begins to break up at 180 mya.
- Africa breaks away from Gandwana at 160 mya.
- Gandwana breaks up in the Cretaceous Era.
- Dinosaurs are extinct around 50 mya.
- Humans appear in the Quatemary Era.



Types of Evolution

- Divergent Evolution
  - The diversification of an ancestral group into two or more species in different habitats.
  - Diverged from a common ancestor.
  - Supported by homologous structures and vestigial structures.
  - Occurs when a population becomes isolated and adapts to different environmental pressures, resulting in speciation. (Allopatric)
- Adaptive Radiation
  - Ancestral species moves into an isolated region.
  - Requires:
    - A change in environment.
    - New resources available.
    - A variety of ecological niches.
- Convergent Evolution
  - The process in which species from different evolutionary lineages come to resemble each other because they have similar ecological roles & natural selection has shaped similar adaptations.
  - Supported by analogous structures.

Differences between Natural and Artificial Selection	
Natural Selection	**Artificial Selection**
Nature selects individuals with favourable variations for better survival in an environment.	Selective breeding of domesticated plants/animals to produce offspring with characteristics that are desirable to humans.
Nature selects the best and favourable variations.	Humans select desirable characteristics.
Always increases the chances of survival.	May not always increase chances of survival.
The environment exerts selection pressure.	Humans exert selection pressures.
Takes hundreds of years for a new species to emerge.	
Operates on a wide scale of natural populations.	
Leads to greater diversity.	Leads to less diversity.
E.g. insecticide resistance, giraffe's long neck, Finches beaks.	E.g. Breeding of wolves → dogs Breeding of cows

Similarities:

- Selection occurs in both.
- Both results in changes to allele frequency.
- Both require variation in a gene pool beforehand.
- Determines which genetic traits pass from one generation to the next.
- Traits must be inheritable.

Sexual Selection

- Mating behaviour in animals.
- Causes sexual dimorphism, in which females and males of a species are distinctly different in appearance and behaviour.
- Certain characteristics on males attracts mates.
    - E.g. Antler size, Tail size, behaviours such as courtship displays.

Natural Selection and Speciation

Allopatric Speciation

1. **Variation**
   A population with variation exists.

2. **Isolation**
   A physical barrier is formed, separating the population in two.
   Gene flow between the two new populations is not possible.
   Each population now has their own gene pool.

3. **Selection**
   Different selection pressures act on both populations.
   This brings a change in allele frequencies in the gene pool.
   Leads to evolution of subspecies.

4. **Speciation**
   Separate gene pools have changed so much over time that they cannot interbreed to produce fertile offspring.
   Two species exist.

Allele Frequencies and What Changes Them

- Allele frequency
  - How often an allele is present in a population.
  - Affected by:
    - Natural Selection
    - Artificial Selection
    - Sexual Selection
    - Mutations
    - Migration/Emigration → Gene flow
    - Genetic Drift
    - Bottleneck Effect
    - Founder Effect

- Genetic Drift
    - Random
    - Every reproductive event result in the inheritance of half of the genes from the father and the other half form the mother. This is controlled by chance.
    - In small populations, some genes/alleles could never be passed down due to chance.
    - Can completely remove genes from a gene pool.
- Bottleneck Effect
    - Occurs when a population decreases dramatically.
    - Low genetic diversity occurs.
    - Can completely remove many genes from a gene pool.
    - E.g. Cheetah population greatly decreased after Ice Age. The surviving males all had genes for high chances of infertility, and this is what was passed down to cheetah generations today.
- Founder Effect
    - Small number of individuals isolate themselves from a parent population.
    - Have a different allele frequency over time due to different selection pressures.