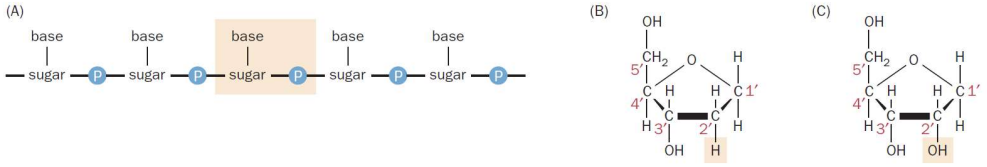
Human Molecular Genetics – Exam Revision

DNA and Chromosomes

DNA Structure

- Large polymers with long linear backbones of alternating residues of a phosphate and a five-carbon sugar; attached to each sugar residue is a nitrogenous base.
- The sugars in DNA and RNA differ, in either lacking or possessing an -OH group at their 2'-carbon positions. In DNA the sugar is deoxyribose (lacking a -OH group), in RNA the sugar is ribose (possessing an -OH group). 3' to 5' is what give DNA its direction.
- The phosphate backbone attaches itself to the 5' carbon, while the bases attach themselves to the 1' carbon.
- The sequence of bases identifies the nucleic acid and determines its function, there are four types found in DNA: adenine (A), cytosine (C), guanine (G), and thymine (T). A small base will pair with a large base to keep the strands roughly the same distance apart. A and T will have 2 hydrogen bonds, and C and G will have 3 hydrogen bonds.



DNA Direction

- DNA has a direction of 5'→3'. The two anti-parallel DNA strands run in opposite directions linking 3' to 5' carbon atoms in the sugar residues.
- This is conventionally abbreviated to give the 5'→3' sequence on only one strand.
- It is usual to describe DNA by writing the sequence of bases on one strand only, in the 5'→3' direction, which is the direction of synthesis of new DNA or RNA from a DNA template

Chromosomes

- We have two types of DNA in our cells: nuclear DNA and mitochondrial DNA. Our cells contain 3×10^9 DNA bases in each nucleus; this DNA is broken up into pieces, each piece forms a chromosome.
- Chromosomes have two fundamental roles: faithful transmission and appropriate expression of genetic information.
- For a chromosome to be copies and transmitted accurately to daughter cells, it requires three types of structural element:
    - A **centromere**, where it is the narrowest part of the chromosome and the region at which spindle fibres attach.
    - **Replication origins**, certain DNA sequences along each chromosome at which DNA replication can be inherited.
    - **Telomeres**, the ends of linear chromosomes that have a specialised structure to prevent internal DNA from being degraded by nucleases.
- A **metacentric** chromosome has two arms that are the same length; a **submetacentric** chromosomes has one short arm (p arm) and one long arm (q arm); an **acrocentric** chromosome has only one arm.

- There are 24 different human chromosomes: 22 autosomes, and the X and Y chromosomes. Autosomes are numbered from largest to smallest with the exception that chromosome 21 is slightly smaller than chromosome 22.
- Most of our cells are diploid, with 2 of each autosome and 2 sex chromosomes; autosome pairs are called homologues. The correct diploid number for humans is 46 chromosomes (23 pairs)
- The Y chromosome is the smallest with very few functional genes (approximately 70) and is most involved with male sexual development. The X chromosomes is very large and gene dense (approximately 900, ~5% of all genes).

Analysing Chromosomes

- Get a tissue sample and grow it in a culture, adding chemicals to stimulate mitosis.
- Incubate for 2 to 3 days.
- Add chemical to stop mitosis in metaphase.
- Transfer cells to a tube and centrifuge to concentrate in layers.
- Transfer to a tube containing fixative.
- Put cells onto microscope slides; add stain to enhance chromosomes.
- Identify and photograph chromosomes.
- Cut out chromosome pictures and arrange into karyotype.

Mitochondrial and Nuclear DNA

- Mitochondria is considered the 'energy factory' of the cell. It generates ATP through oxidative phosphorylation.
- Cells have a variable number of mitochondria depending on energy demands.
- Mitochondria: make their own DNA, RNA, and proteins, have modified genetic code and don't use introns, divide when they need to regardless of cell cycle, not compacted at metaphase and do not appear in a karyotype analysis.
- The human mitochondrial genome consists of a singly type of circular double-stranded DNA that is 16.5 kilobases in length. It contains 37 genes: 2 ribosomal RNAs, 22 transfer RNAs, and 13 polypeptides (proteins), all subunits of enzyme complexes of the oxidative phosphorylation system.
- During zygote formation, the mitochondrial genome is maternally inherited: males and females both inherit their mitochondria from their mother, but males do not transmit their mitochondria to subsequent generations.

Polymerase Chain Reaction (PCR)

- PCR can make millions of copies of DNA and relies on the base pairing of DNA: A-T, G-C
- Need to know part of the sequence at the stand and the end of the region of DNA you want to copy
    - You make primers (short DNA sequences) which will match this sequence
    - This is the only part of the reaction that is specific to the target sequence
    - Primers determine the size and sequence of the PCR product
- Also need:
    - Taq polymerase – thermostable polymerase enzyme
    - PCR buffer – supplied with the enzyme
    - Magnesium – this is needed by the Taq enzyme; a higher Mg^{2+} concentration decreases specificity

    - dNTPs – deoxynucleotides; A, G, C, and T go into PCR product
    - Template DNA – the sequence you are going to amplify the product from
- Three temperature dependent steps, in the following order:
    - Denaturation – heating makes the DNA template single stranded, up to 100^{o}C
    - Annealing – cooling allows primers to anneal to their complementary sequence, 55 ^{o}C to 65 ^{o}C
    - Extension – polymerase extends the DNA sequence from the primers, 72 ^{o}C
- The annealing temperature is the most important temperature as it will determine if the primers bind to the template sequence
- Reaction is carried out in a PCR thermocycler – usually 35 cycles

Sanger Sequencing

- It relies on random inhibition of chain elongation, creating newly synthesised DNA strands of various lengths that can be separated by size. The DNA needs to be in a single-stranded form that will act as a template for making new complementary DNA strain *in vitro* by using a suitable DNA polymerase.
    - it all starts by having a short primer binding next to the region of interest. In the presence of the 4 nucleotides, the polymerase will extend the primer by adding on the complementary nucleotide from the template DNA strand.
    - To find the exact composition of the DNA sequence, we need to bring this reaction to a defined stop that allows us to identify the base of the very end of this particular DNA fragment.
    - This is done by removing an oxygen atom from the ribonucleotide, this is called a dideoxynucleoside. The polymerase enzyme can no longer add normal nucleotides onto the DNA chain.
    - The extension has been stopped and we identify the chain terminating nucleotide by a specific fluorescent dye (4 specific colours).
    - Sanger sequencing results in the formation of extension products of various lengths terminated with dideoxynucleosides at the 3’ end.