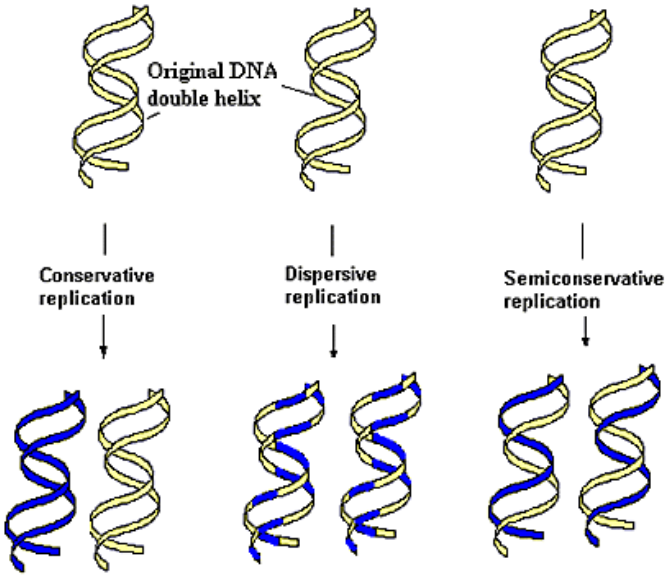
DNA Replication

When/ Why/ How does DNA need to Replicate?

- Replication occurs during Interphase before Cell Division
- Each daughter cell contains an exact copy of parents genetic material- cannot survive of function without DNA
- Accuracy- Allows organisms to reproduce themselves to preserve a species



Possible Models of DNA Replication

- Helicase separates the 2 stands of DNA
- DNA Polymerase picks up free nucleotides from the nucleoplasm, working from 5 prime to 3 prime
- DNA polymerase creates phosphodiester backbone by complimentary base pairing onto the two parent strands which act as templates

How?

- Replication begins at specific sequence known as replication origin
- Helicase enzyme unwinds and unzips the parental DNA, complimentary strands held by weak Hydrogen Bonds
- Whole DNA molecule will have been unwound and replicated by end
- Parent strands act as template, new strands built up from free DNA nucleotides that are abundant in nucleoplasm
- Free nucleotides attach (via Hydrogen Bonds) to exposed parental strands
- DNA Polymerase enzyme joins new nucleotides to each other by strong, covalent phosphodiester bonds- forms sugar-phosphate backbone of new strand
- Each new identical molecule is made up of one new and one original chain from the parental molecule- semi-conservative replication
- Meselon-Stahl 1958

Gene Therapy

Insertion of a new DNA sequence to replace function of a faulty gene that causes genetic disease
Gene therapy can be in two forms; germ- line therapy and somatic cell therapy

Germ- line Therapy

- Germ- line cells are those cells in a eukaryote, from which the gametes are derived i.e. the tissue in the ovaries, which produce ova, or the tissue in the testes from which spermatozoa are developed
- Research involves injecting the ova with replacement gene, or the early developing embryo

Advantages;

- Tissues of the new adult contain new gene, so genetic disorder is cured, and this can lead to an increased chance of that adult producing progency without the defective gene

Difficulties;

- Chance of genome being disrupted: if this occurred the defective gene would still be present and any additional disruption could be passed on to future generations
- There are ethical issues associated with germ- line therapy e.g. engineered babies etc. approach avoided for these reasons

Somatic Cell Therapy

- Somatic cells are all of body's cells with the exception of the gametes
- Therapy attempts to replace defective gene in the cells of the affected tissues

Advantage;

- Not having to correct all of affected tissues, just enough to reduce adverse effects

Adding New Genes into Genome

There are two ways of adding new gene into genome. Either add directly within the body or withdraw tissues and then replaced the treated cells.

DNA of unaffected gene is extracted from donor cell. This fragment of DNA is replicated using Polymerase Chain Reaction (PCR). The target piece of DNA needs to be sequenced which will have a promoter region where copying of the gene will begin and a termination region where the sequence will end. Special primers are added to a heated mixture of DNA. The heat separates the DNA double helix into single strands by breaking hydrogen bonds between the base pairs. Primers attach to the DNA at their target sequences, only a few bases long. Enzyme Taq Polymerase (which is heat resistant archaeal DNA polymerase enzyme) binds to promoter region and starts to make a copy of the gene until it reaches the terminator sequence. New double helix sequences are then heated and the process repeated until as large numbers of copies of the gene are made.

Direct Uptake of DNA- Cystic Fibrosis

Microbiologist have isolated the gene which codes for protein, required for normal mucus production. Malfunction of the lungs is usually the cause of death and poor functioning affects the sufferer most it has been under intense research.

2) Restriction endonuclease cuts at recognition sites producing many smaller fragments. HVR-enzme cuts around to keep HVR intact
3) DNA has (-ve) charge so attracted to anode so is separated into bands. Longer fragments struggle to pass through gel matrix, smaller fragments travel further.
4) Denatured- gel immersed into alkali, which unzips double strand DNA. Gene probes can then bind to unpaired bases on single strands
5) Blotting- Thin nylon sheet with blotting paper on top. Single strands of DNA fragments are drawn up into membrane where they stick
6) Radioactive/ UV gene probe added which binds to single stranded DNA fragments that contain complimentary base sequences. Unbound probes must be rinsed off thoroughly.
7) Radioactive probe exposes film so patterns of dark and light bands are revealed producing a DNA profile unique to each individual

Genetic fingerprinting is used for paternity tests, genetic screening and forensics.