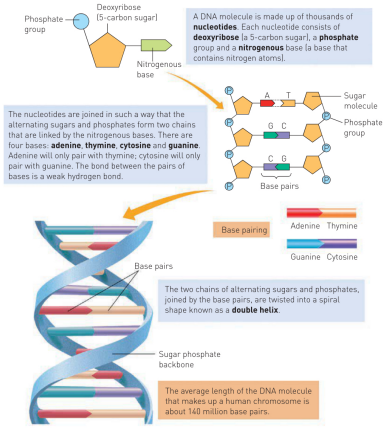
Objective Sheet 5

MUTATIONS AND TECHNIQUES IN BIOTECHNOLOGY **HUMAN PERSPECTIVES CHAPTER 12-13**

1. DESCRIBE THE STRUCTURE OF DNA



2. DEFINE MUTATION AND EXPLAIN THE POSSIBLE SOURCES OF MUTATION

Mutation is a permanent structural altercation in the DNA

Factors affecting the outcome of a mutation
- The type of cell affected (somatic or germ line)
- The type of mutation (e.g. substitution, insertion)
- The extent of the mutation (e.g. point, non-disjunction)
- The area of the gene affected (coding or non-coding)

Mutagen

A mutagen is anything that causes a mutation (i.e. causes a permanent change in a cell's DNA)

- Most mutagens are either chemicals or physical agents such as radiation.
- Some mutations are spontaneous, occurring as a result of errors during DNA replication or repair.

3. EXPLAIN THE DIFFERENCE BETWEEN GENE MUTATION AND CHROMOSOMAL MUTATION

GENE MUTATION
- Are changes in a single gene so that the trait normally produced by that gene are changed or destroyed
- An alteration to a single gene

Point
- Simplest type of mutation
- This involves a change in just one of the bases in a DNA molecule

Lethal recessive
- Complex type of mutation
- Recessive Allen that, inherited in homozygous condition, results in the death of the embryo, foetus or child (is deadly in it's homozygous form)

CHROMOSOMAL MUTATION

A change to the structure and/or number of chromosomes in an organism
- Deletion
- Duplication
- Inversion
- Translocation
- Non disjunction

4. EXPLAIN THE DIFFERENCE BETWEEN A SOMATIC MUTATION AND A GERMLINE MUTATION

SOMATIC MUTATION

- A somatic mutation is a DNA change that occurs in body cells, other than the egg or sperm (germ cells)
- Somatic mutation cannot be passed on to offspring.

GENELINE MUTATION

- A germ line mutation is a DNA change that occurs in the egg or sperm.
- Germ line mutations can be passed on to offspring

5. UNDERSTAND WHAT A POINT MUTATION IS AND IT'S POSSIBLE CONSEQUENCE

POINT MUTATION

- The simplest type of mutation is a point mutation
- This involves a change in just one of the bases in a DNA molecule

Consequence

A point mutation could alter a protein, have no effect at all, or prevent the protein from being produces. Thus if the DNA of a particular gene is altered, the protein for which it codes may be missing or abnormal, just one missing if abnormal protein could have an enormous effect on the entire body

6. LIST THE WAYS THAT A GENE MUTATION CAN OCCUR

CHROMOSOMAL MUTATION

1) **Deletion**

- Part of a chromosome is lost

2) **Duplication**

- A section of chromosome occurs twice.
- This may happen if part of a chromatic breaks off and joins on to the wrong chromatid

3) Inversion

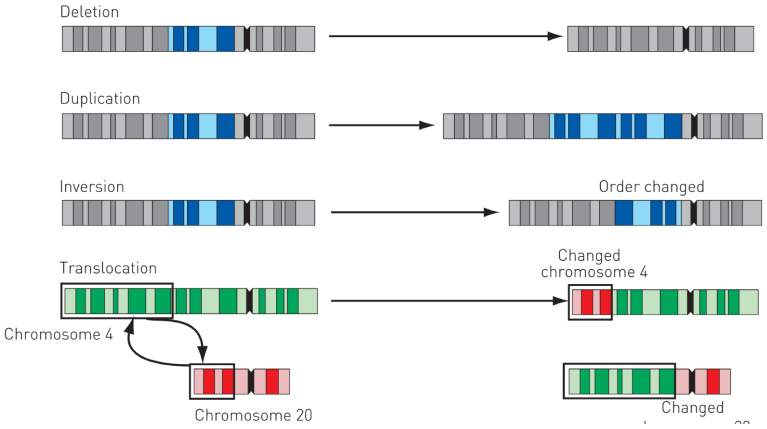
- Break occurs in a chromosome and the broken piece joins back in, but the wrong way around.
- This changes the order of the gene on the chromosome and may disrupt the painting of homologous chromosome during meiosis

4) **Translation**

- Part of a chromosome breaks off and is re-joined to the wrong chromosome

5) **Non disjunction**

- During meiosis, a chromosome and one daughter cell has an extra chromosome and one daughter cell has one less than the normal chromosome number



7. DESCRIBE THE BIOTECHNOLOGY TECHNIQUES INCLUDING

■ DNA sequencing

DNA sequencing is the precise order of nucleotides in a small of DNA

- DNA is denatured by heating to a high temperature to form single stranded DNA
- Primer is attached to the DNA template and stands is equally divided into four tubes. DNA polymerase (tag polymerase) is also added, as well as four DNA nucleotides (DNTPs)
- Inside each tube a different DNA nucleotide is placed, but with hydrogen synthetically replacing the hydroxyl group on the deoxyribose sugar

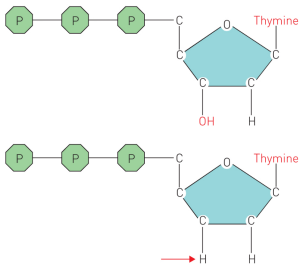
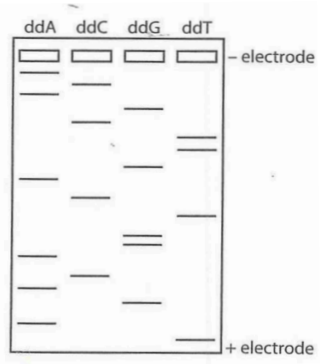


Figure 13.2 DNA is synthesised from four deoxynucleotide triphosphates. One of them, deoxythymidine triphosphate (dTTP), is shown in the top formula. Shown below is the synthetic nucleotide dideoxythymidine triphosphate (ddTTP).



- When one of these dideoxynucleotides is inserted into the replication chain, the DNA sequence ing stops at this point
- As the process is repeated several times. The DNTPs are inserted at different points in the DNA chain, forming different lengths of DNA strands
- Using gel E, fragments of different lengths are separated for each of the four nucleotides. Short fragments travel furthest etc.

Uses of DNA sequencing
- Determining if a person will inherit a disease
- Parental test
- Forensics
- Evolutionary biology

■ DNA profiling

- Everyone's DNA is unique (except for identical twins), half being inherited from the mother and half from the father.
- The unique nature of DNA provides the basis for DNA profiling - also known as DNA fingerprinting.

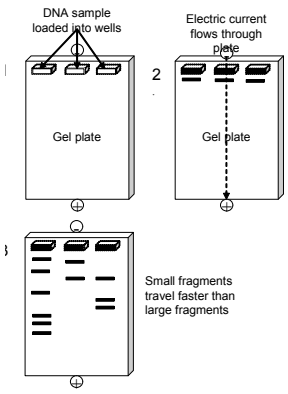
Creating a DNA profile
- A DNA profile can be created from a very tiny sample of DNA (e.g. from the saliva or a fingerprint on a glass). A profile can even be established from very old or damaged DNA.
- From a single sample many copies can be produced using a technique called the polymerase chain reaction (PCR).
- The segments of DNA is cut at specific points
- The DNA fragments differing is sizes are then separated using gel electrophoresis

<u>Summary</u>
1. Amplify the DNA (PCR).
2. Cut up the DNA and separate the fragments (Gel E) DNA profile/fingerprint. (bands of DNA)
3. Sequence the bands into individual bases. (DNA sequencing)

Uses of DNA profiling
- Identifying an individual identify
- Determining their percentage
- Detecting genetic variation and/or mutation

Gel Electrophoresis

- A process for separating fragments of DNA into different sizes using an electric current
- The samples to be tested are injected into small wells on a sheet of porous, jelly like material
- An electric current is passed through the gel and the fragment are drawn towards the positive end
- DNA is negatively charged, thus it is attracted to the positive end and the small fragments travelling faster than the larger ones



Uses for Gel Electrophoresis

- For finding an individual's DNA profiling or DNA fingerprint.
- Tracing ancestry
- Forensics science
- Identification of hereditary disease.

Polymerase Chain Reaction (PCR)

- The polymerase chain reaction (PCR) is like a biological photocopier. It is used for creating multiple copies of a specific section of DNA from a sample (DNA amplification).
- PCR is useful when only small amounts of DNA are available for analysis.

Denaturing

- Denaturing involves heating the DNA to 96C* to separate the two strands.
- A heat stable DNA polymerase (Taq Polymerase) is used.

Hybridisation

- Primers (short synthetic DNA fragments) are added to the DNA.
- The primers bind to complimentary base sequences on the separated DNA strands.
- The primers act as starting points for the replication of new DNA molecules.
- Temperature 55-65C*

DNA synthesis/elongation/extension

- DNA polymerase (Taq) is added.
- Starting at the primer, the DNA polymerase reads the DNA code and builds a complementary strand of DNA, by adding complimentary nucleotides.
- This occurs at 73C*
- Each cycle takes 3-5 minutes.

Uses of polymerase chain reaction

- Gene analysis
- Evolutionary studies
- Forensic science
- Detection and diagnosis of disease

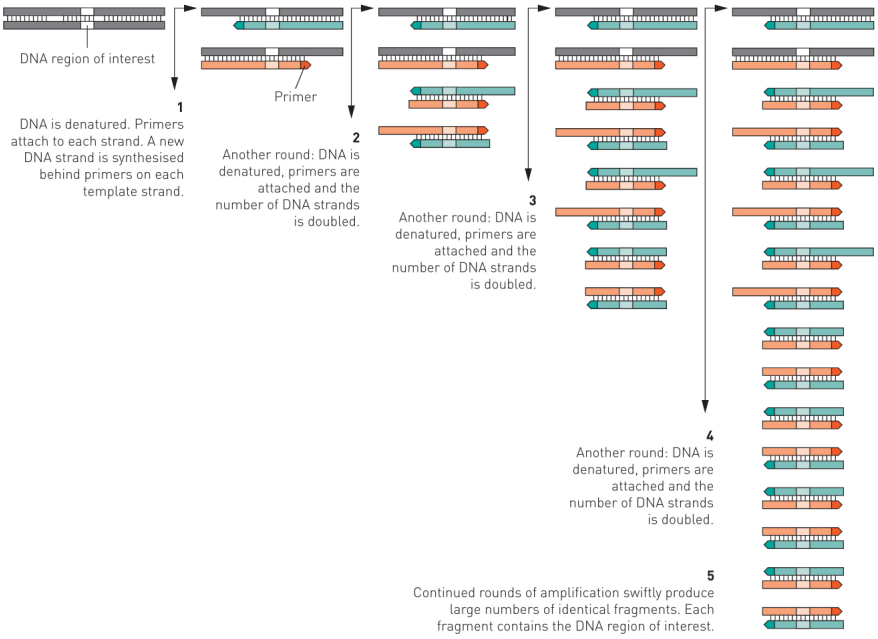
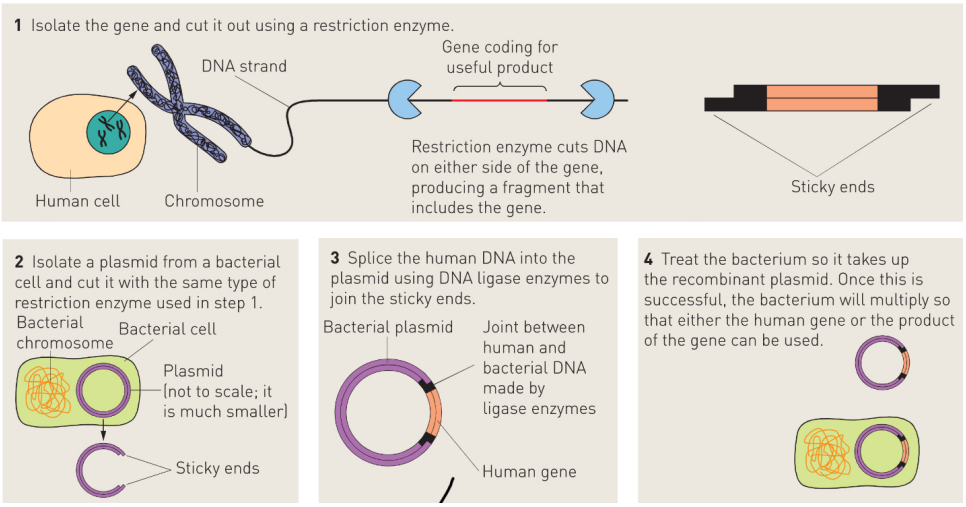


Figure 13.5 A diagrammatic representation of the polymerase chain reaction

Recombinant DNA



1) Isolate the gene of interest and cut it out using restriction enzymes. Make a fragment of DNA that includes the gene
2) Isolate a plasmid from a bacterial cell and cut it with the same type of restriction enzymes (a plasmid is a small, circular double stranded unit if DNA, that can replicate independently if the nucleus DNA)
3) Gene of interest is spliced into the plasmid and now called recombinant DNA
4) Cloning if the vector -> large amount of DNA to insert into host cells
5) It is integrated back into the bacteria cell
6) Bacteria cell (host cell) will then reproduce the foreign protein

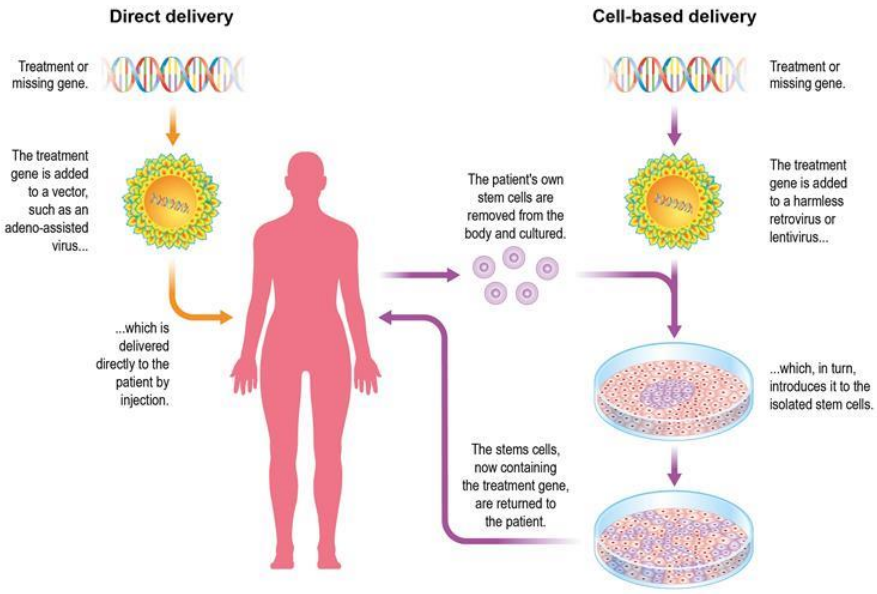
Used of Recombinant DNA
- Production of insulin
- Production of human growth hormone
- Production of factor VIII
- Vaccine

Cell Replacement Therapy

Cell replacement therapy is to replace damaged faulty cells in order to reduce the symptoms of the disease

- A technique that involve growing cells in controlled laboratory conditions, it's a technique that has been used for many years in medical research and has been the forerunner for the new technologies of cell replacement therapy and tissue engineering.
- These techniques aim to replace damaged and diseases cells with healthy ones
- Situations ideal for cell replacement therapy is any disorder involving loss of, or damage to, normal cells

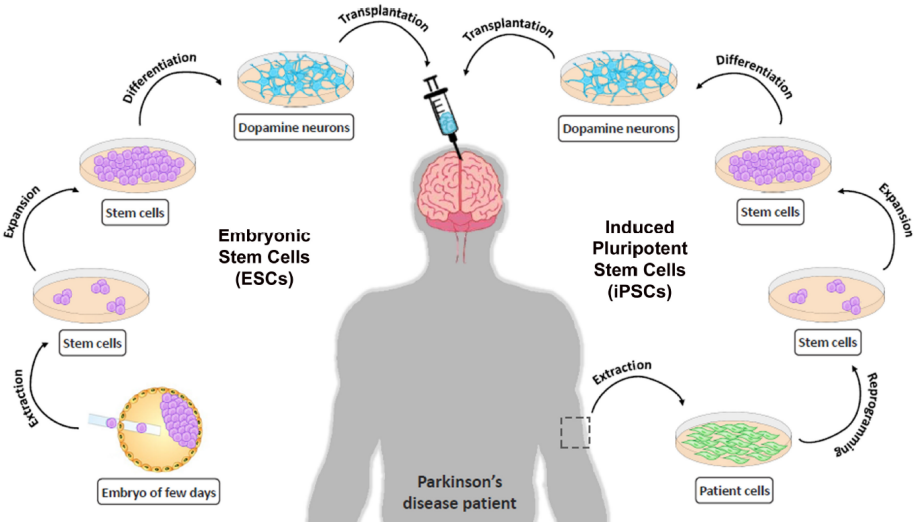
Steps
- Fertilised egg matures until it reaches blastocyst stage (8 cells)
- Blastocyst cells are harvested
- Cells taken from inner cell mass
- Cells stimulated with growth factors
- Cells mature into adult nerve cells
- Cells to be cloned
- Nerve cell are then injected back into the patient
- New cell replace damaged nervous tissue
- Symptoms of disease reduced



STEM CELLS
Undifferentiated Cells that are capable of repeated biotic divisions for long periods of time and, given the right conditions, can differentiate into specialised cells. These characteristics make them ideal for producing replacement tissue

Embryonic Stem Cells (ESCs)
- Cells removed from embryo whilst still pluripotent
- Source of embryos - IVF
- Expansion- grow in-vitro
- Differentiation into dopamineric neurons
- Transplantation into patients brain

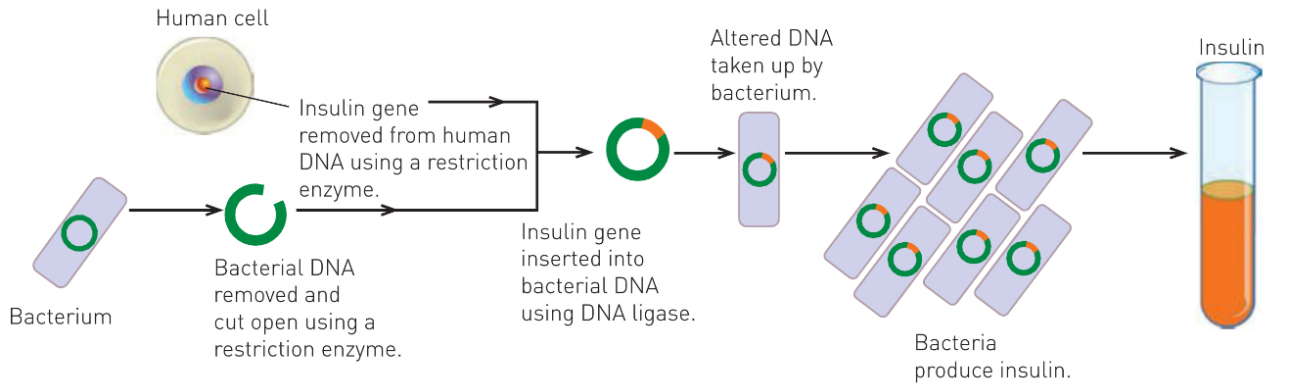
Induced Pluripotent Stem Cells (iPSCs)
- Somatic cells removed from patient
- These cells are reprogrammed using retroviruses to deliver genes
- They are one pluripotent
- Expansion- grow in-vitro
- Differentiation into dopamineric neurons
- Transplantation into patients brain



In vitro cloning: Made in the laboratory
- PCR

In vivo: made in a living cell/thing
- Recombinant DNA

8. DESCRIBE HOW INSULIN/ VACCINES ARE PRODUCED USING RECOMBINANT DNA



1) Insulin DNA gene is cut from the surrounding DNA using restriction enzymes
2) Plasmid DNA is cut with the same restriction enzyme
3) Insulin DNA is incorporated into plasmid DNA and a bacterial cell is transformed
4) Bacteria contains plasmid DNA survives antibiotics treatment and are culture to generate clones. Clones produce large quantities of desired gene production, insulin

9. DEFINE GENE THERAPY. LIST SOME GENETIC DISORDERS THAT ARE CURRENTLY BEING RESEARCHED AND/ OR USED IN GENE THERAPY

GENE THERAPY

The treatment of disease by replaying, manipulating or supplementing non functional genes with cells and tissues. Replaying faulty genes with healthy ones.

Genetic disorders that are currently being researched in gene therapy

- Cystic fibrosis
- Huntington's disease

10. OUTLINE SOME ETHICAL CONCERNS WITH USE IF GENETIC ENGINEERING AND RECOMBINANT DNA

Risks

- Allergic reaction
- Effects of embryo
- Vaccines using autism

Ethical concerns

- Were its tested
- Playing god
- How its made
- Killing babies

Benefits

- Herd immunity
- Prevention of diseases when travelling