Biology Unit 2 Gene Technology

Microarray Technology: Consider these problems -

1. In every population variation exists in genetic base sequence. So an individual's base sequence can determine how their body will respond to certain drugs, how can we determine this?
2. Genes can also be switched on (expressed) or off, which can result in disease. So how can we compare genes expressed in a normal tissue with those expressed in cancerous tissue?

To solve these problems, we can use advanced technology. A microarray (DNA chip or GeneChip) are miniature spotting tiles (can be glass or silicone) that have thousands of DNA probes attached to it. The DNA probes are added to the chip robotically and information about the each one is kept on a computer file.

First problem: (gene sequence variation)

- Individuals DNA is amplified by PCR
- Digested using restriction enzymes
- Made single stranded
- Label added (florescent or chemiluminescent)
- Labelled DNA added to a microarray

The single stranded DNA will only bind on the spots where complementary probes are located. Due to the labels we can digitally analyse the chip to see where binding has occurred. This info tells us an individual's base sequence for the gene of interest, allowing for appropriate medication to be given.

Second problem: (gene expressing profiling)

- All mRNA is extracted from the tissue of interest (e.g. from normal & cancerous tissue)
- Reverse transcriptase used to make cDNA copy of each mRNA
- cDNA's labelled
- Added to microarray and analysed

This allows the researchers to which genes are 'up-regulated' and 'down-regulated' in cancerous cells compared with normal cells.

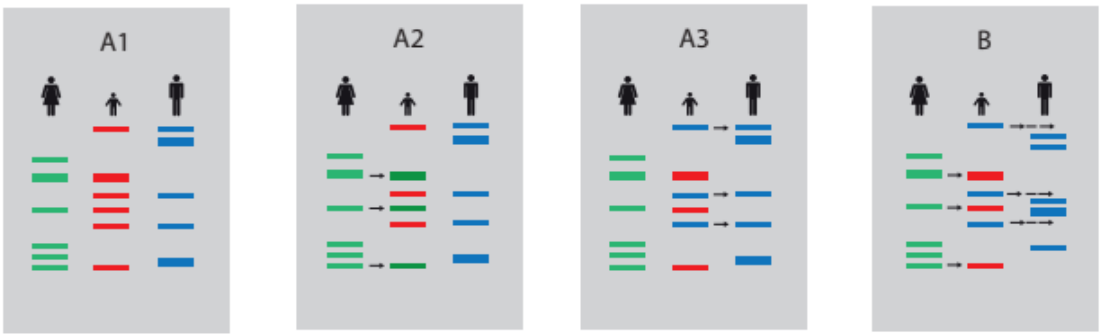
Genetic Fingerprinting

Technique for analysing and comparing the DNA of individuals. To produce a genetic fingerprint:

- Sample DNA is cut into smaller fragments using restriction enzymes
- Fragments added to an agarose gel and separated by size, using gel electrophoresis
- DNA made single stranded and copied onto a nitrocellulose sheet
- Labelled DNA probes added to the plate and bind by base pair complementarity
- A detection system used to show where the binding has occurred

The probability of two people (other than identical twins) having the same profile when 10 or more markers are used is virtually impossible. This allows the profiles produced to be used for:

- Crime scene analysis
- Paternity disputes
- Immigration disputes
- Conservation (to establish species membership & assess genetic variation in populations)



Which of the fingerprints prove paternity and which disprove paternity?

A1 - A3 prove paternity, as all bands present in the child's fingerprints are present in either the males or the females fingerprint.

B disproves paternity, as those bands that the child does not have in common with the mother are not found in the males fingerprint.

Genetic Sequencing

An organism's genome can be defined as all of the genetic material (DNA) which the organism has in one set of chromosomes. There have been techniques developed to determine the order of the bases in the genomes, this is called genome sequencing. Knowledge of the base sequence enables the primary structure of the protein encoded by that gene to be worked out. Then the secondary, tertiary and quaternary structure can be worked out.

Useful for:

- Determining causes of disease & tailoring treatment
- Investigating evolutionary relationships
- Genetic testing & forensics

Huge advantages in sequencing tech have reduced the cost and time involved in genome sequencing, since 'next-generation sequencing' began to be used.

Social & ethical implications of gene sequencing:

The advent of direct-to-consumer gene sequencing products (e.g. 23andme kit) available from at least one high street retailer has social implications. These kits enable consumers to obtain a report on various aspects of their genome, including whether they carry genes which may increase their risk of developing diseases later in life & how they metabolise various common medications. There are concerns about releasing this information to individuals and who else might request access the information.

Pharmacogenetics

Is the tailoring of drug treatments to individuals, based on their genotype. Microarray technology & gene sequencing have contributed to this field, by providing information on an individual's genetic makeup.

Due to genetic variation people can respond differently to certain drugs. In some ethnic groups, such as Africans and African Americans there is an increased incidence of the 'ultra-rapid' metaboliser genotype. An individual's genotype with regard to the alleles involved can be determined via microarray so that a safe and effective dose can be prescribed.

Another branch of pharmacogenetics involves cancer genotypes. Knowledge of the specific mutations in the cancerous cells and which genes are being up- or down-regulated can help in determining the best chemotherapy treatment.

Designer Drugs:

With increased knowledge of the genetic variation underlying diseases & drug responses, researches can now explore treatments which may only work in a subgroup of patients (e.g. Herceptin - a cancer drug). The development of 'designer drugs' will make side effects less common & ensure that the right dosage is prescribed first time.

Therapeutic Genetic Modification

1. Use of genetically modified viruses to treat cancer: Herpes virus to treat malignant melanoma
   - It could no longer make a protein that enables the virus to reproduce
   - The viral gene was disrupted
   - It had a human cytokine gene inserted into it which triggers an immune response to the infected cells
2. Use of GM viruses to treat bacterial infections:
   - Viruses have been genetically modified to improve the efficiency of antibiotics against bacterial infections.
   - Some of which target bacterial DNA, but bacterial DNA have a defence mechanism which helps repair DNA targeted by the antibiotics.
   - The GM viruses disrupt the DNA repair action, allowing the antibiotic to get to work
3. Gene therapy (First developed in 1972)
   - 2011 - reported it had successfully been used to treat haemophilia B (a sex-linked blood clotting disorder caused by a recessive allele on the X-chromosome)
   - Patients injected with viruses acting as healthy vector, which delivered the healthy gene to the cells in the liver
   - Gene therapy has been less successful in the treatment of cystic fibrosis. One reason being the target cells in the lungs are regularly renewed and so repeated doses of gene therapy need to be given.