

## Investigative techniques in genetic medicine

Peripheral blood leucocytes are typically used to study chromosomes and DNA. However, almost any growing tissue can be used e.g. skin fibroblasts, amniocytes or cells from chorionic villi. DNA may also be extracted from archived histopathological material and Guthrie blood spots, although analysis may be limited.

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### Samples for genetic analysis

#### Blood samples

- Karyotype: 5-10ml blood in lithium-heparin blood tube
- DNA: 5-10ml blood in EDTA blood tube

#### Skin biopsy

- Punch biopsy (3-5mm) placed in transport medium for fibroblast culture
- Can be used for karyotype, biochemical assays and DNA analysis

#### Amniocytes and chorionic villi

- See below

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### DNA analysis

The lymphocytes from a 10ml blood sample yield approximately 300ug of DNA, sufficient for multiple DNA analyses. DNA can be stored frozen for very many years.

#### Polymerase Chain Reaction (PCR)

- Used to amplify a DNA sequence of interest (see figure)
- Two oligonucleotide primers are used which are complementary to the DNA sequence of interest
- Firstly, the double stranded DNA is denatured by heat into single-stranded DNA fragments
- The reaction is then cooled to allow annealing (binding) of primers to the target DNA sequence
- Heat-stable *Taq1* DNA polymerase then extends the primers along the template target sequence
- After one cycle there are 2 copies of double-stranded DNA, after two cycles there are 4 copies and so on
- Typically a PCR reaction involves 25-30 cycles, hence allowing millions of DNA amplifications

#### DNA Sequencing

- Di-deoxy sequencing allows identification of the exact nucleotide sequence of DNA
- The region of interest is amplified by a PCR reaction (see above). This builds a new DNA chain complementary to the region of interest
- The reaction is carried out in 1 tube, containing all four nucleotides and all 4 di-deoxy nucleotides (adenine, cytosine, guanine, thymine).
- The di-deoxy nucleotides are incorporated into the growing chain and where incorporated stop the addition of any further nucleotides
- The fragments are electrophoresed and the DNA sequence is determined by fluorescence
- DNA sequencing can be used to identify point mutations, small deletions and insertions.

#### Southern Blotting

- DNA is cut into fragments using a restriction enzyme that recognises a specific DNA sequence
- The fragments are separated according to size by gel electrophoresis and transferred (blotted) to filter paper
- A probe is then used to bind to its complementary sequence on

- the blot and can be identified by autoradiography
- Southern Blotting requires relatively large amounts of good quality DNA and is time-consuming

#### Multiplex Ligation-dependent Probe Amplification (MLPA)

- This is a relatively new, high resolution method to detect copy number variation in genomic sequences eg large gene deletions and duplications
- Often gene analysis now involves DNA sequencing and MLPA to identify different types of mutation
- MLPA can also be used instead of FISH for the analysis of telomeres

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### Chromosome analysis

#### Karyotyping (chromosome analysis)

- Samples are cultured
- Cell division is then stopped in metaphase
- Chromosomes stained with Giesma i.e. G-banding
- Alternating light (euchromatin) and dark (heterochromatin) bands are characteristic for each chromosome pair using light microscopy
- The resolution of G-banding is approximately 5Mb
- See figure

#### Fluorescent In Situ Hybridisation (FISH)

- Technique used to detect the presence of specific DNA sequences on chromosomes
- Uses fluorescent probes and light microscopy
- See figure

#### Telomeric FISH

- FISH analysis using probes specific for telomeres (chromosome ends).
- Improves sensitivity of microdeletions or other cryptic rearrangements as most telomeres are G-band-negative

#### Haematological malignancies and solid tumours

- Haematological neoplasms and paediatric solid tumours can be characterized by acquired genetic rearrangements detectable using karyotype analysis
- Used to provide information about diagnosis eg Philadelphia chromosome (9;22 translocation) in CML, for prognosis and remission

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### Molecular Cytogenetics

Technology using molecular DNA analysis has recently been developed to determine genomic copy number.

#### Array-CGH (comparative genomic hybridisation)

- Patient and control DNA are given different fluorescent labels and hybridised to arrays containing thousands of genomic clones
- The fluorescent signals are compared to determine whether there is a change in genomic copy number (deletions or duplications) of patient DNA relative to control DNA
- The resolution is much greater than routine karyotyping and FISH
- However, it cannot detect apparently balanced rearrangements
- A number of variants have been identified with as yet unknown significance. Therefore, this technology is still being used with caution in determining results.

- In the future, this technology is likely to significantly increase the ability to diagnose chromosome abnormalities as a cause of mental retardation and congenital defects.
- See ref. Vissiers et al

#### Quantitative fluorescence polymerase chain reaction (QF-PCR)

- QF-PCR is used to test for gene dosage, ie the number of copies of a given gene present in a sample.
- Examples of its use includes testing for aneuploidy of whole chromosomes eg 13, 18, 21, X, Y in a CVS or amniotic fluid
- Markers of DNA from the sample are amplified, labelled with fluorescent tags and measured by electrophoresis.

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### Genetic testing & Prenatal Diagnosis

#### Amniocentesis

- Under ultrasound guidance, needle inserted transabdominally through uterine wall for aspiration of amniotic fluid
- From 15 weeks gestation
- Miscarriage risk 1% above normal population risk
- Can be used for fetal karyotype, biochemical assays and DNA analysis

#### Chorionic villus sampling (CVS)

- Under ultrasound guidance, needle inserted transabdominally through uterine wall for biopsy of chorionic villi
- From 11 weeks gestation
- Miscarriage risk 1% above normal population risk
- Can be used for fetal karyotype, biochemical assays and DNA analysis

#### Pre-implantation genetic diagnosis (PGD)

PGD is a form of genetic diagnosis performed prior to implantation.

- The patient's oocytes are fertilized in vitro and, at the 8 cell stage of embryogenesis, a single cell is removed and analysed for the genetic condition in question.
- The diagnosis itself can be carried out using several techniques, eg FISH and PCR

#### References

Vissiers LEM, de Vries BBA, et al. Array-based comparative genomic hybridisation for the genomewide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 2003; 73:1261-70.

*FIGURES*

FISH

PCR

CGH