Techniques to study chromosome aberrations.

In the second of our series we will look at the key techniques used to examine for chromosome rearrangements in human cells and also, how to make sense of the data which is generated.

The starting point for all cytogenetic analysis is to prepare chromosomes for viewing down a microscope. As described in the Summer 2016 edition of the BNTVA magazine all nucleated cells in our bodies contain genetic material, meaning we could sample any cell from our bodies to determine our chromosome constitution. Blood cells are the most easily sampled for this purpose and have the added advantage of being able to enter a 'growing phase' in the laboratory by the addition of a stimulant known as a mitogen. Lymphocytes (white blood cells) respond to this mitogen by progressing through their cell cycle (Figure 1) toward mitosis which is the phase where a cell begins to divide into two daughter cells and importantly, is the phase where the cellular genetic material is sufficiently condensed to be visible down a microscope as discrete chromosomes. A mitotic blocking agent can then be added to 'arrest' the growing cells before actual division enabling the 'fixation' of lymphocytes at a stage where their chromosomes are in this condensed 'metaphase' state. The duration of the cell culture and the timing of addition of the arresting agent are optimised to collect an adequate number of predominantly first-division metaphase cells, thereby ensuring any chromosome aberrations subsequently identified are representative of the blood initially sampled.

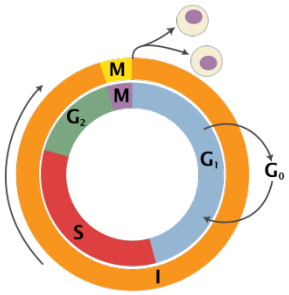
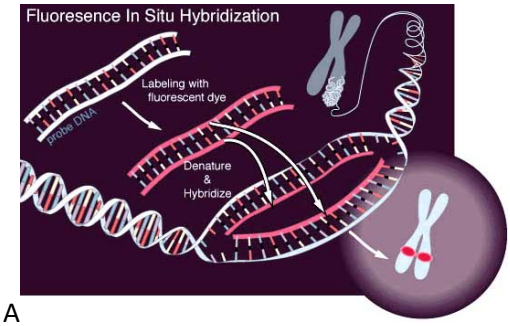


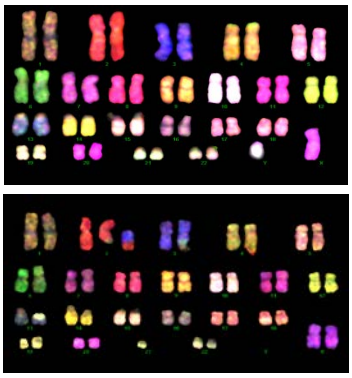
Figure 1: Cells have distinct stages (G1, S (DNA synthesis), G2 and M (mitosis)) of growth collectively termed as the cell cycle. Each cell will proceed through a full cell cycle and then divide into two daughter cells. Chromosomes are most visible (and therefore useful for analysis) in the metaphase stage of mitosis (M).

Fixed cells are then placed on microscope slides and stained using a variety of techniques each of which can reveal different aspects of the chromosome morphology. For the NCCF-Cytogenetics Study the predominant technique will be fluorescence *in situ* hybridisation (FISH), including multiplex (M)-FISH. The principle of FISH relies on the fact that DNA is double stranded (see Article 1) and that each strand is complementary in its base-pair sequence. What this means is that under conditions in the laboratory, double stranded DNA can be 'melted' or denatured into two single strands and when the conditions are altered, these two strands can come back together again by matching the complementary base pairs to re-form double stranded DNA. If, into this mix, we add a section of single stranded DNA that is also complementary but which has been chemically modified with a fluorescence label or tag, then sometimes this fluorescent probe will 'hybridise' rather than the original, resulting in a fluorescently labelled sequence of DNA which we can visualise using a fluorescence microscope (Figure 2A). By preparing probes that span whole chromosomes and by

using spectrally different fluorescent colours then we can distinguish multiple different chromosomes by their unique paint colour (Figure 2B top). We can determine if the structure of each painted chromosome is normal or exchanged in some way by assessing if the coverage of paint is continuous down the whole length of the chromosome (Figure 2B top) or, if there is a colour-switch (Figure 2B bottom). The colours of any rearranged chromosome then determines the origin of chromosomes involved. M-FISH represents the most advanced version of this chromosome painting technique and also requires specialised software to process the multiple different combinations of fluorescence colour and probe combinations.



A



B

Figure 2: (A) Principle of the FISH technique. The diagram shows the use of a single probe to label a specific region on one chromosome however probes can also be generated to 'paint' whole chromosomes. (B) M-FISH karyotype showing all human homologous chromosome pairs uniquely painted with distinct colours in a normal cell (top). Chromosome exchanges are detected as colour-switches, in this example; chromosomes 2, 3, 13 and 21 are rearranged (bottom).

There are many types of structural aberration visible with whole chromosome FISH techniques including stable exchanges e.g. translocations, insertions and, unstable exchanges e.g. dicentrics, centric rings, acentric fragments. All of these categories can be recorded and an assessment made as to whether the same aberration is present in all cells (constitutional) or in just a fraction of the cells analysed. For non-constitutional changes i.e. those which have been acquired throughout our lifetime (see Article 1) we can calculate their frequency of occurrence in each individual sampled, provided, a sufficiently large number of cells are analysed. For evaluative purposes however we need to repeat this process across a pre-defined sample population. In the NCCF-Cytogenetic study this population comprises 50 test families, selected based on knowledge that they are veterans (or a family member of a veteran) who participated at a nuclear test site, together with 50 matched control families. It is only by comparing the frequency of aberrations between these two similar, yet distinct populations, that any statistical relevance of the findings can be determined. An example of this would be where the average frequency of chromosome translocations derived from 50 test veterans is compared to the same averaged metric in the control veterans using appropriate statistical methods.