Advance Laboratory Techniques
Lecture 6

Fluorescence in situ hybridization (FISH)

FISH (fluorescent *in situ* hybridization) is a cytogenetic technique developed by biomedical researchers in the early 1980s[1] that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes.

FISH is a powerful technique used in the detection of chromosomal abnormalities. The high sensitivity and specificity of FISH and the speed with which the assays can be performed have made FISH a pivotal cytogenetic technique that has provided significant advances in both the research and diagnosis of haematological malignancies and solid tumours.

From a medical perspective, FISH can be applied to detect genetic abnormalities such as characteristic gene fusions, aneuploidy, loss of a chromosomal region or a whole chromosome or to monitor the progression of an aberration serving as a technique that can help in both the diagnosis of a genetic disease or suggesting prognostic outcomes.

FISH is essentially based upon the principle that exploits the ability of single-stranded DNA to anneal to complementary DNA. In the case of FISH, the target is the nuclear DNA of either interphase cells or of metaphase chromosomes affixed to a microscope slide, although FISH can also be performed using bone marrow or peripheral blood smears, or fixed and sectioned tissue. Once fixed to a microscope slide, the desired cells are hybridized to a nucleic acid probe. This anneals to its complementary sequence in the specimen DNA and is labelled with a reporter molecule which is either an attached fluorochrome, enabling direct detection of the probe via a coloured signal at the hybridization site visualized by fluorescence microscopy, or a hapten that can be detected indirectly.

Genetic aberrations

Although the DNA replication process is remarkably accurate, human cells are estimated to contain double-strand breaks in cell cycle If left unrepaired, such damage results in the loss of chromosomes and/or the induction of cell death. If imprecisely repaired, the damage leads to mutations and chromosomal rearrangements. These DNA double-strand breaks (which may result in gene malfunction) are considered to be critical primary lesions in the formation of chromosomal aberrations and can occur in both somatic and germ-line cells.

Fortunately, cells devote significant resources to the repair of DNA double-strand breaks, inducing several cellular responses including DNA repair via recombination-dependent DNA replication, cell cycle checkpoint activities associated with biochemical pathways resulting in the delay or arrest of cell cycle progression, and the triggering of apoptotic pathways. The mitotic, or spindle assembly, checkpoint is a mechanism that arrests the progression to anaphase to ensure accurate chromosome segregation. Mitotic arrest is induced when errors occur in the spindle structure or in the chromosomal alignment on the spindle. Loss of mitotic checkpoint control is a common event in human cancer cells, which is thought to be responsible for chromosome instability with various defects causally implicated in tumourgenesis. However, how mitotic arrest contributes is not well defined.

Mutations can occur in the genomes of all dividing cells as a result of misincorporation during DNA replication or through exposure to exogenous mutagens such as ionizing radiation or endogenous mutagens

Probes

The potential of almost all applications of in situ hybridization is greatly enhanced by multicolour detection of simultaneously hybridized probes. This is particularly useful when structural chromosome aberrations involving different chromosomal regions are to be diagnosed, or when several numerical aberrations should be detected in parallel.9 One of the most important considerations in FISH analysis is the choice of probe. A wide range of probes can be used.

There are broadly three types of probe, each with a different range of applications: whole-chromosome painting probes; repetitive sequence probes and locus-specific probes.1 Chromosome 'painting' refers to the hybridization of fluorescently labelled chromosome-specific, composite probe pools to cytological preparations, which allows the visualization of individual chromosomes in metaphase or interphase cells and the identification of both numerical and structural chromosomal aberrations in human pathology with high sensitivity and specificity.

An example of the power of FISH is that of the genetic translocation t(12;21)(p12;q22) in acute lymphoblastic leukaemia (ALL), which was first detected through the use of FISH with chromosome painting probes.

Whole chromosome painting is available for every human chromosome, allowing the simultaneous painting of the entire genetic complement in 24 colours.

Repetitive sequence probes hybridize to specific chromosomal regions or structures that contain short sequences which are present in many thousands of copies. Examples of this probe type are Pan-telomeric probes targeting the tandemly repeated (TTAGGG) sequences present on all human chromosome ends or centromeric probes that target the a and b satellite sequences, which flank the centromeres of human chromosomes. centromere-specific probes particularly suitable for the detection of monosomy, trisomy and other aneuploidies in both leukaemias and solid tumours.3 A centromere-specific probe for chromosome 8 has been used to detect trisomy by FISH in newly diagnosed patients with a myelodysplastic syndrome or acute myeloid leukaemia (AML)

locus-specific probes : Probes of this classification are particularly useful for detecting structural rearrangements such as specific chromosomal translocations, inversions or deletions in both metaphase and interphase For example, a locus-specific probe for the BCR (breakpoint cluster region) gene at 22q11.2 detected with a green flurochrome and a locusspecific probe for the ABL (Abelson oncogene) gene at 9q34 detected with a red fluorochrome will appear as a bright yellow spot (the combination of green and red fluorochromes) in leukaemia cells when viewed via fluorescence microscopy, characterized by the BCR/ABL fusion gene, resulting from t(9;22)(q34;q11.2)

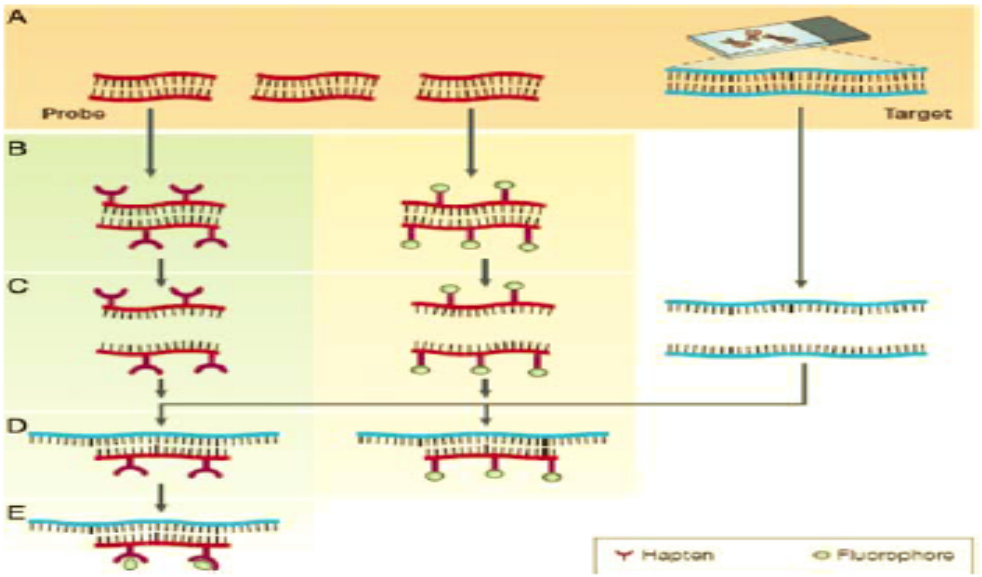

Figure 1. The principles of fluorescence *in situ* hybridization. (**A**) The basic elements are a DNA probe and a target sequence. (**B**) Before hybridization, the DNA probe is labelled indirectly with a hapten (left panel) or directly labelled via the incorporation of a fluorophore (right panel). (**C**) The labelled probe and the target DNA are denatured to yield single stranded DNA. (**D**) They are then combined, which allows the annealing of complementary DNA sequences. (**E**) If the probe has been labelled indirectly, an extra step is required for visualization of the non fluorescent hapten that uses an enzymatic or immunological detection system. Finally, the signals are evaluated by fluorescence microscopy. Adapted from Speicher and Carter.[35] Reprinted by permission from Macmillan Publishers Ltd: *Nat Rev Genet* 6: 782–792, copyright 2005.

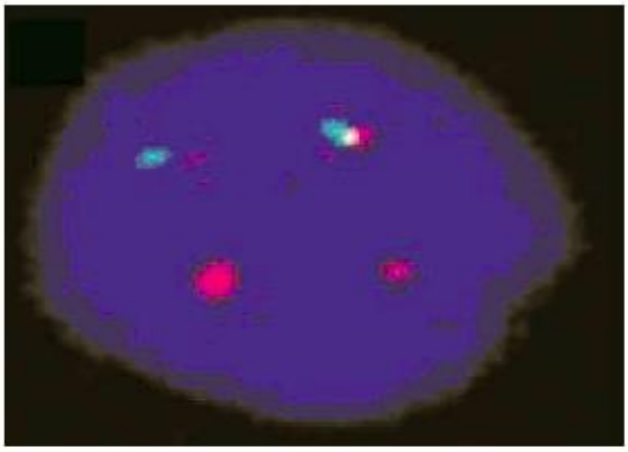

Figure 4. Interphase FISH on a nucleus using an Exta signal probe to detect the *BCR/ABL* translocation. The green signal indicates the presence of the *BCR* gene, red signals indicate the presence of the *ABL* gene and the red green fusion (yellow) signal confirms a *BCR/ABL* translocation. The extra red signal confirms this is not a false positive result. Adapted from Primo *et al.*[54] Reprinted by permission from Macmillan Publishers Ltd: *Leukemia* 17: 1124–1129, copyright 2003.

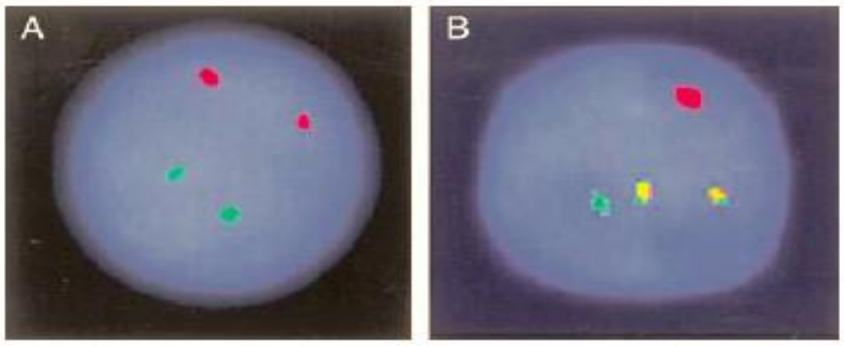

Figure 5. Interphase FISH using dual fusion probes on two separate nuclei to detect. (**A**) Normal nuclei and (**B**) nuclei containing *BCR/ABL* double fusions indicated by green red fusion (yellow) signals. Adapted from Dewald *et al* by permission of the American Society of Hematology.[31]

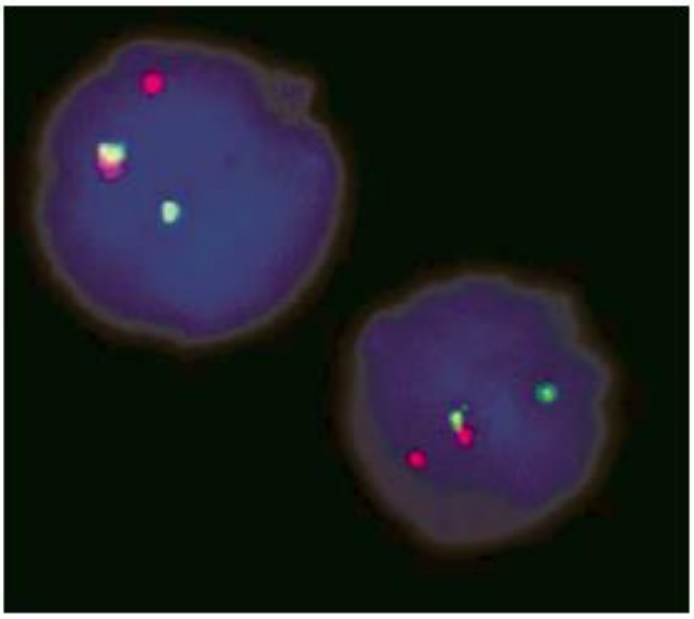

Figure 6. Interphase FISH on bone marrow nuclei containing the translocation t(11;19)(q23;p13) using a dual colour split apart probe. Green red fusion (yellow) signals indicate a normal cell. Separate green and red signals indicate the presence of translocations. Adapted from Kearney with permission from Elsevier.[1]

Table 1. Examples of FISH applications

Diagnostic	Research
The identification of specific chromosome abnormalities	The identification of new non random abnormalities (by M FISH or SKY)
The characterization of marker chromosomes	Gene mapping
Interphase FISH for specific abnormalities in cases of failed cytogenetics	Identification of regions of amplification or deletion by CGH
Monitoring disease progression	The identification of translocation breakpoints
Monitoring the success of bone marrow transplantation	The study of 3D chromosome organization in interphase nuclei

Adapted from Kearney with permission from Elsevier.[1]