Fluorescent in-situ Hybridization Technique "FISH"

Fluorescence *in-situ* hybridization (FISH) is a cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity. It is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes.

Principle:

The fluorescence in situ hybridization technique involves a fluorescently labeled DNA probe being hybridized to genomic DNA sequences, and can be used to physically map the specific site on the chromosome or tissue.

Methodology:

1. **Chromosome preparation**

   Metaphase chromosome spread can be prepared using spindle inhibitor such as colchicine to arrest dividing cells during mitosis. Hypotonic (0.075M KCl) treatment followed by fixation (methanol: Acetic acid in 3:1 ratio) will fix the chromosomes. Dropping of fixed cell suspension on glass slides followed by chemical ageing in 2X SSC or physical ageing at 90 degree Celsius make the slides ready for FISH. Before hybridization, pre-treatment with 0.05% pepsin will enhance the accessibility of the probe and reduce the amount of cytoplasm.

2. **Probe labelling**

   For probe labelling, pure DNA preparation is required and a fluorochrome must be incorporated into the DNA probe. DNA probes can be labelled by enzymatic procedures such as nick translation, random priming or by polymerase chain reaction (PCR). For nick translation, 3'-5' exonuclease and 5'-3' polymerase activity of DNA polymerase I cause a single strand nick and subsequently, a specific fluorescently labelled nucleotide incorporate into the nicked strand using non nick stand as a template. Labelling by PCR involves a standard PCR reaction with labelled nucleotide.

3. **Hybridization and detection**

Following pre-treatment, hybridization carried out under optimal conditions for the annealing of the probe to specific target site. Mix the probe with cot 1 or salmon sperm DNA and applied on slides for hybridization. Hybridization between probe and target DNA takes place during the incubation period of 16-24 hrs at 37 degree Celsius. This incubation time can vary depending upon the probe used.

Detection of probe permits the visualization of target DNA sequences. In direct labelled reaction in which fluorochrome conjugated nucleotide used can directly observed under fluorescent microscope at specific wavelength. In an indirect labelled reaction in which biotin or digoxigenin labelled nucleotides are incorporated into the DNA requires incubation of slides in reporter molecules like avidin of anti-digoxigenin conjugated with fluorochrome. The detection method for biotinylated probes employs avidin-fluorochrome conjugates whereas for digoxigenin labelled probes employ antidigoxigenin-fluorochrome conjugates.

4. **Chromosome counterstaining and fluorescence microscopy**

In addition to signal detection, the DNA of the chromosomes or nucleoli must be counterstain for visualization. Typical fluorochromes used in conjunction with FISH include Hoescht 33258, DAPI and Propidium iodide. It may necessary to vary the concentration of counterstain in order to optimize signal detection.

Types of FISH probe:

a) **Gene-specific probes**

Gene specific probes are useful for mapping genes on chromosomes. Gene specific probe target the specific DNA sequence within the chromosome or tissue sample. Example of such probes includes bacterial artificial chromosome (BAC) and yeast artificial chromosome (YAC) probes and cosmids. These probes have proven useful particularly in the study of micro deletion syndrome where the absence of a gene often goes undetected by conventional banding methods.

b) **Repetitive sequence probes**

Repetitive sequence probes bind to regions that are rich in repetitive base pair sequences. Examples of such probes include centromeric and telomeric probes. Centromeres contain A-T rich tandem repeats, where as telomere are recognized by short repetitive sequence i.e. TTAGGG. Centromeric probes have applications in the identification of marker chromosomes and numerical chromosome abnormalities in interphase nuclei. Telomeric probes and subtelomere specific probes are commonly used to identify cryptic chromosomal translocations such as those occurring in case of unknown mental retardation.

c) Chromosome painting probe

Chromosome painting probes contain sequences that are specific to either a single chromosome (i.e. whole chromosome painting probe) or an arm of a chromosome (i.e. chromosome arm painting probe). After hybridization, one or more chromosomes of interest are light up in different colour, which are dependent on the type of particular fluorochrome used. This probe is particularly useful for identifying chromosome arms that are involved in translocations, as well as for marker chromosomes and ring chromosomes.

d) Whole genomic DNA probe

Whole genomic DNA probes are used for FISH based technique comparative genome hybridization (CGH). They can be used to detect genomic imbalance in tumour genomes by combining tumour and normal DNA to analyse gains and losses.

Applications of FISH Technique:

- localization of repetitive sequences, transposons, and transgenes
- Identification and characterization of sex chromosomes
- Characterization of interspecific hybrids and chromosome set in manipulated Finfish
- Assignment of genetic linkage groups to specific chromosomes (Genome Mapping)
- Identification of pathogens in cultured shellfish, finish, and wastewater generated by aquaculture