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Fluorescent in Situ Hybridization (FISH)

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SUMMARY

FISH is a molecular cytogenetic technique in which florescent probe used to bind only those parts of the chromosome with a high degree of sequence complementary. "The method for identifying the piece of DNA within a genome is called fluorescence in situ hybridization (FISH)". The technique is used for detecting and locating a specific DNA sequence on a Chromosome. It can use to visualize specific cytogenetic abnormalities like chromosome translocation, chromosome deletion and amplification. This article summarized the concept of FISH, with protocol and application having their advantages.

INTRODUCTION

FISH was developed in early 1980. The fluorescent probe used in FISH is nucleic acid labeled with fluorescent group and it binds to specific DNA /RNA sequences. It helps to identify or understand when and where a specific DNA sequences exist in cell by detecting fluorescent group. Fluorescent microscopy helpful to find out where the probe bound to chromosome and binding quantitatively detected by flow cytometry. FISH is an important technique for understanding a variety of chromosomal abnormalities and different type of genetic mutations. This technique provide a new era for researchers to visualize and map the genetic material in an individual cell in the portion of genes at specific region.

Protocol of FISH

Two major and very important elements required for FISH. One is the probe and second is target sequence.

There are different types of probe used in this process.

- Centromere Probe
- Locus specific probe.
- Whole Chromosome probe.
- Telomere Probe.

Centromere Probe – Alpha and Satellite probes generated from repetitive sequences found in centromere. Centrome regions are stained brighter.

Locus Specific Probe – Locus-specific probes target a specific gene sequence of interest. These probes can be used to determine whether a gene is deleted, amplified or present in a normal copy number as well as translocation of specific probe.

Amplification is usually determined by comparison to the number of centromeres in the same cell.

It also called as gene amplification Probes.

Whole Chromosome Probe –

Whole chromosome probes are collections of all smaller probes, each binds to a different sequence along the length of a given chromosome. Using multiple probes labeled with a mixture of different fluorescent dyes, scientists are able to label each chromosome in its own unique color.

The resulting full-color map of the chromosome is known as a spectral karyotype.

It is particularly useful for examining the chromosomal abnormalities, such as when a piece of one chromosome is attached to the end of another chromosome.

Telomere Probe -

Telomere probe is a PNA FISH probes provide a simple technique for the detection of human telomere sequences by FISH using fluorophore-labelled PNA probes.

The technique provides appropriate quantitation as the fluorescence intensity is directly correlates to the length of the telomeres. Specific to the 300Kb locus at the end of specific chromosome.

I Sample Preparation –

If test sample is material such as cell line, chromosome preparation etc,. It is necessary to fix the preparation for 10 minute in a mixture of methanol and acetic acid in the ratio of 3: 1 after its application onto the microscope slide (Coating, imprint or cytospin).

Fixation mixture prepare instantly before the use, after fixation proceed to co-denaturation and hybridization. If the sample is paraffin slice, necessary to cut FFPE (Formalin fixed paraffin embedded) tissue into 5um slices on positive charge glass slides then bake at 56^0 c overnight, deparaffinize and preterit material.

II Hybridization and Co-denaturation:-

Now apply the probe in a quantity to the test sample and the cover with cleaned cover glass slide. Slide denature at 73$\pm$ 1^0c for 1-5 min. After that incubated slide overnight at 37^0c in moist chamber.

III Wash off unbound probe:-

Remove the cover glass and immerse the preparation in washing solution I (0.3% NP-40) heated up to 73$\pm$ 1^0C for 3-5 sec. shake glass slightly with prepare solution. Transfer glass on washing solution II (2X ssc/ 0.1% NP-40) and shake again for 3-5 sec then incubate for 30 sec. Dry slightly by attaching the edge of the glass to an absorbent pad and let it dry naturally. Apply mounting medium containing DAPI. The main aim is to stain the nuclei like to be able to observe them using a fluorescence microscope. Cover using a cover glass and examine under the fluorescence microscope.

IV Solution prepare

Denaturation solution:- 49ml formamide, 7ml 20X SSC, 14ml purified water to be modified and maintain the pH 7-8, store at 2-8^0c, heat up to 73$\pm$ 1^0 C before use.

Washing Solution I :- (0.4X SSC/ 0.3% NP-40):- 20 ml 20X SSC, 3ml NP-40 , add purified water up to 1liter of total volume, modify pH to 7.0, store at laboratory temperature; heat up to 73$\pm$ 1^0 C before use.

Washing Solution II ;- (2X SSC/0.1% Np-40) : 100ml 20X SSC, 1ml NP- 40 add purified water up to 1 liter total volume, modify pH to 7.0, store at laboratory temperature.

Applications:-

- Morphological and population structure of microorganisms.
- Pathogen profiling abnormal gene expression.
- Gene expression profiling in embryonic tissue.
- Unique FISH patterns on individual chromosomes, chromosomal aberrations useful tool in gene mapping and characterization of chromosomal aberrations.
- Numberous diseases can be diagnosed using FISH include prader-willi syndrome, Chronic Myelogenous leukemia, angelman syndrome analysis of 21 chromosome, X and Y can also identify oligozoopermic individuals at risk.
- It also provide the important molecular information in the context of cell morphology.
- Novel oncogenes identification of species.

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